ILLUMINATING MICROBIAL CONTRIBUTIONS TO SOIL CARBON CYCLING DYNAMICS USING HIGH RESOLUTION STABLE ISOTOPE PROBING

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ILLUMINATING MICROBIAL CONTRIBUTIONS TO SOIL CARBON CYCLING DYNAMICS USING HIGH RESOLUTION STABLE ISOTOPE PROBING

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Terrestrial carbon (C) represents the largest active global C pool. Microbes are estimated to mediate ~80-90% of soil C-cycling, yet the complexities of the soil ecosystem have limited our ability to disentangle specific microbial contributions. We are still grappling with the importance of microbial community structure and function to ecosystem processes. Diversity in microbial communities may not be important to contemporary ecosystem processes, but maintaining microbial diversity may prove to be of the utmost importance to prevent disruption of ecosystem processes as the climate and environmental conditions change. Here, I use high resolution stable isotope probing (HR-SIP) to examine the variations of C substrate use in soil when composition and timing of amendment additions are varied. I found that decomposition of C substrates occurs in a successional pattern characterized by the use of low molecular weight compounds at an early stage, followed by the use of high molecular weight compounds later in time. Specifically, xylose (low molecular weight) was metabolized quickly (within 7 days) while cellulose was metabolized more slowly (2+ weeks). This succession of decomposition was accompanied by transitions in the microbial community: fast-growing spore formers responded quickly and assimilated xylose-C, while slow-growing microorganisms responded slowly and assimilated cellulose-C. I also found the amount of cellulose decomposed largely depended on the composition of accompanying amendments but not on the

phylogenetic composition of cellulose utilizers. While the total amount of cellulose decomposed varies with differences in amendment composition, the pattern of cellulose decomposition over time (from beginning to end of incubation) is the same, albeit with varying magnitude, regardless of amendment composition. Together, these results demonstrate that cellulose decomposition is not disrupted by varying amendment conditions or phylogenetic affiliation of cellulose responders. This means that biodiversity maintains ecosystem function as environmental conditions fluctuate and a decrease in biodiversity could result in unforeseen suspension or loss of ecosystem functions.

BIOGRAPHICAL SKETCH

Ashley Campbell grew up in Troy, North Carolina. She was the first in her family to go to college. She completed her Bachelors degrees in Biology and Chemistry at Guilford College in Greensboro, North Carolina. At Guilford College, she was advised by Drs. Melanie Lee-Brown (biology) and Robert Whitnell (chemistry). After college Ashley was a research assistant at Harbor Branch Oceanographic Institute studying marine sponge microbial communities under the guidance of Dr. Peter McCarthy. Once Ashley was hooked on the microbiology bug, Melanie Lee-Brown inspired her to continue her education by pursuing a Ph.D. in Microbiology. In August 2009 Ashley began her graduate career at Cornell University in the Department of Microbiology. She joined Dr. Daniel Buckley's lab in the Department of Crop and Soil Science (field of Microbiology) because she admired Dr. Daniel Buckley's mentorship and meticulous approach to science. Dan Buckley led by example, cultivating Ashley's approach to science and mentorship. For three consecutive summers (2011-2013) Ashley worked as the course coordinator for the Microbial Diversity course at Marine Biology Laboratory (MBL) in Woods Hole, Massachusetts with Dan Buckley and Dr. Stephen Zinder. At MBL, Ashley was fortunate enough to meet a multitude of scientists, established and new. She discovered she loved multidisciplinary science and working in teams while at MBL. She hopes to move towards research that integrates multidisciplinary approaches with a collaborative team of scientists. After her Ph.D., Ashley will begin a postdoctoral position at Lawrence Livermore National Lab (LLNL) under the guidance of Dr. Jennifer Pett-Ridge. She is excited about the prospect for collaboration with her appointment at LLNL.

Dedicated to my mom, Cindy Barton, who always supported me.

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Introduction

1.1 Background

The terrestrial environment is important to global carbon (C) cycling and climate change. It contains an estimated 2,500 gigatons (Gt) of C in its active carbon pool, more than the sum of C in the atmosphere (800 GtC), surface ocean (1000 GtC), and plant biomass (550 GtC) (Trivedi et al., 2013). When 11 global C models were compared, predictions for the atmosphere and ocean environments all predicted an increase in flux of CO_2 into the atmosphere and ocean, respectively (Friedlingstein et al., 2006). However, these same 11 global C models were inconsistent in their predictions for the terrestrial environment (Friedlingstein et al., 2006), with some models predicting that soils could be a net source while other models predict soils to be a net sink of C (Friedlingstein et al., 2006; Piao et al., 2013). The terrestrial environment is difficult to parameterize resulting in inconsistencies of terrestrial C models. Furthermore, comparisons of models are confounded by the numerous parameter settings and interacting effects.

The terrestrial environment is difficult to parameterize in part due to the overwhelming complexity of soil and in part due to our inability to predict land use changes and their associated effects. Land use changes can have large impacts on soil C (Six et al., 2006). For example, pristine soils converted to intensive cultivation exhibit a loss of ~25% of the original C stored (Six et al., 2006). Models have been shown to be better predictors of the real world when their parameters are calibrated with experimental data sets (Wieder et al., 2013; Luo et al., 2015). In

order to mitigate the inconsistencies of global C models for the terrestrial environment we need to continue to disentangle the multitude of environmental parameters through experimental approaches. Parameters that are suggested to have the strongest effect in increasing the predictability of terrestrial C models are soil C retention time, microbial contributions and water content/hydrology (Keenan et al., 2013; Wieder et al., 2013; Niu et al., 2014; Luo et al., 2015).

Soil is composed of biological, chemical, and physical components that are intimately intertwined in a network of complex interactions. The stochastic nature of complex soil interactions contributes to our difficulty in disentangling what governs the fate of C (Helgason et al., 2014). Retention time of soil C hinges on its lability and accessibility, which are a function of biological and physical-chemical processes (King 2011). Microbial communities are key regulators of soil organic matter (SOM) dynamics; as such, soil C retention time is intimately tied to microbially mediated processes. Shifts in microbial community structure and function play an important role in determining the rates of C loss from soil (Six et al., 2006). Many factors that shape soil microbial community structure have been identified but there are still unaccounted factors influencing microbial community structure (You et al., 2014). Understanding microbial C utilization under a range of conditions is the key to better understanding soil C dynamics (Helgason et al., 2014; Dungait et al., 2012).

1.2 The functional role of microbial community structure in ecosystem processes

Microbes are estimated to be responsible for nearly 80% of soil C cycling (Coleman, Crossley 1996; Nannipieri et al., 2003), yet it remains unclear how soil microbial structure, that is, the genetic diversity and abundances, and functioning drive soil C transformation and turnover (You et al., 2014). Changes in soil microbial community structure are predicted to alter the activity of microbial communities, thus altering soil biological and physiochemical processes (Waldrop, Firestone 2006; You et al., 2014). Elucidating microbial contributions to soil C-cycling are key to better understand global C-cycling and are crucial in determining how shifts in the microbial community structure may lead to changes in soil processes (Weand et al., 2010; You et al., 2014).

There are two hypotheses for the effects of microbial structure on ecosystem processes: (1) functional redundancy (or equivalency) hypothesizes that widespread capability to use organic matter should minimize the effects of shifts in microbial community structure on biogeochemical processes; (2) functional dissimilarity hypothesizes that variations in microbial community structure will be reflected by differences in the ability of the community to carry out specific processes or change the rate of specific processes (Leff et al., 2012; Strickland et al., 2009). Functional dissimilarity has been demonstrated for N cycling processes (Balser, Firestone 2005; Cavigelli, Robertson 2000; Leff et al., 2012), but this phenomenon is suggested to be a characteristic of processes that are performed by a limited set of microbial taxa (Schimel et al., 2005; Leff et al., 2012). Processes such

as C mineralization are considered so common, and heterotrophic microorganisms so diverse, that microbial community structure is expected to have little bearing on the rate at which organic C compounds are decomposed (Leff et al., 2012). However, there is differing evidence to this hypothesis that suggests microbial community structure is important to the rate of C mineralization during the decomposition process (Waldrop et al., 2000; Strickland et al., 2009; Fierer et al., 2007; Nemergut et al., 2010; Philippot et al., 2010; Leff et al., 2012); although, these studies do not directly test whether changes in microbial community structure cause differences in decomposition (Leff et al., 2012).

1.3 Microbial community structure and function

There is particular interest in what facilitates or hinders microbial communities in the mineralization of C. The ability of the soil microbial community to access and metabolize C substrates is influenced by many factors including (but not limited to) physical protection/aggregation, moisture content of the soil, pH, temperature, frequency and type of land disturbance, soil history, mineralogy, litter quality, and N quality and availability (Gessner et al., 2010; Sollins et al., 1996; Torn et al., 2005; Trumbore 2006; Schlesinger 1977). Furthermore, rates of metabolism are often measured without knowing the identity of the microbial species specifically involved in the cycling of the measured process (Nannipieri et al., 2003). Therefore, the first step in teasing out this central problem is to identify microbial groups responsible for the measured process and understand the relationship between community structure and function (O'Donnell et al., 2001).

Fresh organic matter inputs shape the microbial community structure, which ultimately govern the fate of C (Fontaine et al., 2003). A study that varied the identity and diversity of C amendments added to soil microcosms found quantitative impacts on the rate of cellulose degradation (Orwin et al., 2006). One study used three different plot treatments (one with litter removal, one not manipulated, and one with the addition of the litter removed from the other plot) to investigate how litter-driven changes in bacterial community structure contributed to decomposition in tropical soils (Leff et al., 2012). They could not attribute differences in decomposition rates to differences in the microbial community structure, ultimately supporting the functional equivalence hypothesis (Leff et al., 2012). There are several lines of evidence supporting the hypothesis that structurally distinct microbial communities have nearly identical C utilization patterns (Buyer et al., 2002; Waldrop et al., 2000; Ekschmitt, Griffiths 1998; Brant et al., 2006). However, a study that did a reciprocal exchange of litter type and microbial inoculum under controlled environmental conditions revealed differences in community structure can account for ~85% of the variation in litter C mineralization - suggesting functional dissimilarity in decomposition processes (Strickland et al., 2009). Both Leff et al. (2012) and Strickland et al. (2009) observed differences in the rate of decomposition and the microbial community structure between the treatments, yet only Strickland et al. (2009) identified microbial community structure as a significant explanation to the variation in decomposition rates. The inconsistency of the importance of microbial community structure on decomposition rates illustrated by these studies is likely a result of the quality of the

litter addition; Leff et al. (2012) investigated the effects of native litter, varying only the amount of litter, while Strickland et al. (2009) investigated the effects of non-native litter on microbial communities, thus maximizing shifts in the microbial community structure. Taken together, there is supporting evidence for both the functional equivalence hypothesis and the functional dissimilarity hypothesis. As such, the discrepancies of the importance of microbial community structure to function remain unresolved.

Variation in individual processes does not confer variation in the decomposition of organic matter as a whole. Decomposition of organic matter is considered an "aggregate" process (Schimel et al., 2005; Leff et al., 2012), meaning that it consists of multiple individual processes. In the study of litter-driven changes of bacterial community structure, Leff et al. (2012) found differences in microbial community structure had little bearing on variations in organic matter decomposition rates. However, when decomposition of individual C substrates was assessed in these same soils, bacterial community structure accounted for substantial variation in the metabolic responses (Leff et al., 2012). Thus, microbial community structure may be important to individual processes, but it remains moot as to whether it is important to decomposition of organic matter and other ecosystem functions.

1.4 Importance of microbial biodiversity to ecosystems

Comparisons of studies examining the relationship between community structure and function are convoluted by the multitude of environmental factors that vary between environments, making it difficult to draw conclusions. Still the

question remains, does microbial community structure matter on an ecosystem scale? Biodiversity and ecosystem function studies have determined that both the identity and diversity of macroorganisms control the functioning of ecosystems (Cardinale et al., 2012), yet, the importance of community structure for microorganisms with regards to ecosystem scale functioning is debated (Schimel, Schaeffer 2012).

While variations in microbial diversity may or may not alter ecosystem-scale functioning (discussed above in greater detail) contemporarily, microbial diversity may be of the utmost importance in the face of changing environments. It is suggested that increased phylogenetic diversity confers an increase in an ecosystem's resilience by enabling ecosystem functions to be maintained under varying environmental conditions (Allison, Martiny 2008; Doak et al., 1998) as seen in grasslands (Tilman 1996). Niche partitioning occurs when organisms competing for the same resource occupy slightly different ecological niches and/or their means of resource acquisition. These niches can be environmental variables such as temperature or pH. Thus, as environmental conditions change, niche partitioning within the microbial community can preserve ecosystem functions. Furthermore, a decrease in microbial diversity resulting, for instance, from poor land management practices could confer a decrease in ecosystem resilience, thus suspending ecosystem function in the occurrence of unfavorable climate and environmental conditions.

Many processes require microbial species that work in cooperation with one another in order to metabolize substrates in a mutually beneficial manner

(syntrophy). Syntrophic relationships are important to processes such as the breakdown of pollutants (Pelz et al., 1999; Gieg et al., 2014), methanotrophy (Øvreås et al., 1998), and cellulose degradation (Kato et al., 2005). Some of these processes occur at faster rates when microbes work in collaboration, whereas, some processes do not occur unless all cooperative microbial members are present. In some cases syntrophic relationships couple elemental cycles, where the loss of function for one elemental cycle results in the loss of the other (Burgin et al., 2011). The ecosystem scale importance of elemental cycles coupled via syntrophic relationships is currently unknown (Burgin et al., 2011). Should syntrophic relationships prove to be important to ecosystem functions, a decrease in biodiversity could impair ecosystem function if members important to these syntrophic relationships are lost.

The importance of the relationship between phylogenetic and functional diversity in soil microbial communities to terrestrial ecosystem C-cycling is unclear (King 2011). A study that looked at phylogenetic and functional diversity in desert and non-desert soils found that one of the sampled cold desert soils had the highest metagenomic richness but nearly the lowest taxonomic richness (Fierer et al., 2012). The trend usually observed demonstrates an increase in functional diversity with increasing phylogenetic diversity. However, the findings by Fierer et al. (2012) suggest this trend is not always true and that specific microbial community membership should be considered when assessing the specific functionality of a soil microbial community.

1.5 Approaches to studying microbial communities

The classical approach to understanding microbial function is to culture microorganisms from the environment and measure their physiological capabilities in the laboratory. The advent of culture-independent marker gene based methods revealed that culture-dependent methods were capturing less than one percent of the global microbial diversity (Hugenholtz et al., 1998). To date few bacterial groups are in pure culture (relative to the known diversity of microbes), limiting our ability to assess microbial function on a community scale.

Culture-independent techniques have enabled us to measure microbial community diversity and functionality. Noah Fierer and Rob Knight led the charge on charting microbial diversity using culture-independent sequencing approaches and their approaches have been pivotal in changing how we analyze microbial community data sets (e.g. Caporaso et al., 2010; Hamady et al., 2008; Lozupone, Knight 2005; Lauber et al., 2009). These analyses have been crucial in identifying and cataloging microbial diversity and correlating diversity with metadata of the various environmental parameters such as pH (Fierer, Jackson 2006). However, detection of discrete taxa does not provide evidence that those taxa are contributing to the community function (Krause et al., 2014). Taxa detected in gene-based analyses could be dead, dormant, or just extracellular DNA (Lennon, Jones 2011; Blagodatskaya, Kuzyakov 2013). Although the taxa detected can be considered part of a microbial seed bank from which different traits can be resuscitated (Lennon, Jones 2011), obscuring a community's active function and potential function.

Stable isotope probing (SIP) is a means of linking microbial identity to function in a culture independent manner. SIP approaches begin by adding a stable isotope to an ecosystem, incubating over time, and then identifying microbial activity through detection of the isotope in biomarkers. The presence of the stable isotope in biomarkers can single out taxa that are actively contributing to a function while accounting for non-active members (no isotope in biomarkers) of a community (Bodelier et al., 2013). SIP has been used to elucidate the microbial contributions to many biogeochemical processes (Chen, Murrell 2011). Most of the processes studied to date are performed by a limited subset of microorganisms and analyses are made easier by targeting a diagnostic functional genetic marker. However, C-cycling has proven to be more recalcitrant to study because it is performed by a wide diversity of microorganisms and, to date, there are no functional genetic markers to aid in analysis. In the research presented here, I have developed an unprecedented high resolution stable isotope probing (HR-SIP) approach in an effort to tease apart the microbial contributions to soil C-cycling.

1.6 Summary

Disentangling complex interactions in soil presents a special challenge and we are still struggling to understand the importance of microbial community structure and function to ecosystem processes. What we know to date has been limited by the resolution of our methods. In this dissertation I couple next generation sequencing and DNA-SIP to elucidate microbial contributions to soil C-cycling with an emphasis on cellulose decomposition dynamics. These studies aim to reveal how C substrates are utilized within a microbial community based on

identity of substrate, time of sampling, and amendment composition, concentration and timing. In chapter two, I contrast the use of xylose and cellulose by a soil microbial community over time. Then, in chapter three, I compare the use of cellulose by a soil microbial community under different amendment compositions. Finally, in chapter four I explore the use of cellulose by a microbial community when the concentration and timing of nutrient additions vary.

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2 Chapter 2: High resolution DNA-SIP reveals distinct microbial community functional groups for xylose and cellulose decomposition in soil

2.1 Abstract

We describe a high-resolution approach for identifying microbial contributions to soil carbon cycling dynamics using nucleic acid stable isotope probing coupled with next generation sequencing, herein high resolution-stable isotope probing (HR-SIP). We amended soil microcosms with a mixture of model carbon (C) substrates and inorganic nutrients similar to plant biomass. For each treatment a single C constituent, xylose or cellulose, in the C substrate mixture was substituted for its ^{13}C-labeled equivalent. Xylose and cellulose were chosen to represent labile soluble C and polymeric insoluble C, respectively. Microcosm DNA was interrogated for ^{13}C incorporation at days 1, 3, 7, 14 and 30. Incorporation of ^{13}C from xylose into microorganism DNA was observed at days 1, 3, and 7, while incorporation of ^{13}C from cellulose peaked at day 14 and was maintained through day 30. Of 5,940 OTUs detected, a total of 49 and 63 unique OTUs assimilated ^{13}C from xylose or cellulose into DNA, respectively. Xylose assimilating OTUs were more abundant in the soil community than cellulose assimilating OTUs, while cellulose OTUs demonstrated higher ^{13}C-label incorporation than xylose OTUs. ^{13}C-xylose assimilating OTUs exhibited a dynamic pattern of assimilation, manifested by a succession from *Firmicutes*, to *Bacteroidetes*, to *Actinobacteria*. ^{13}C-cellulose assimilating OTUs included members of the poorly characterized phyla *Verrucomicrobia* and *Chloroflexi*.

2.2 Introduction

Excluding live plant biomass, there are 2,500 petagrams of carbon (C) stored in soils worldwide which accounts for ~80% of the global terrestrial C pool (Trivedi et al., 2013; Amundson 2001; Batjes 1996). Fungi, archaea, and bacteria degrade organic C from plants in soil. This C is returned to the atmosphere as CO_2 or remains in the soil as humic substances, which can persist up to 2,000 years (Yanagita 1990). Respiration of plant biomass C produces 10 times more CO_2 than anthropogenic emissions on an annual basis (Chapin 2002). Global changes in atmospheric CO_2, temperature, and ecosystem nitrogen inputs are expected to impact primary production and C inputs to soils (Groenigen et al., 2006) but it remains difficult to predict the response of soil processes to anthropogenic change (Davidson et al., 2006). Terrestrial C predictions from current climate change models are inconsistent on the magnitude and direction (source or sink) of terrestrial C, while models for atmospheric and oceanic C predictions are congruent (Friedlingstein et al., 2006). The inconsistencies of terrestrial C model predictions reflect how little is known about soil C cycling and could be improved by elucidating the relationship between dissolved organic C and microbial communities in soils (Neff, Asner 2001).

Microorganisms mediate an estimated 80-90% of C cycling in soil (Coleman, Crossley 1996; Nannipieri et al., 2003). The heterogeneous nature of soil ecosystems presents a special challenge in understanding microbial processing of nutrients in soils. Soils are biologically, chemically, and physically complex. The complex characteristics of the soil environment drive microbial community composition, diversity, and structure (Nannipieri et al., 2003). Confounding factors such as

physical protection/aggregation, moisture content, pH, temperature, frequency and type of land disturbance, soil history, mineralogy, N quality and availability, and litter quality all affect the ability of the soil microbial community to access and metabolize C substrates (Sollins et al., 1996; Kalbitz et al., 2000). Further, rates of metabolism are often measured without knowing the identity of the microbial species involved (Nannipieri et al., 2003), leaving the importance of community membership to maintaining ecosystem functions unknown (Allison, Martiny 2008; Nannipieri et al., 2003; Schimel, Schaeffer 2012). Litter bag experiments have shown that the community composition of soils can have quantitative and qualitative impacts on the breakdown of plant materials (Schimel 1995). Reciprocal exchange of litter type and microbial inocula under controlled environmental conditions reveals that differences in community composition can account for 85% of the variation in litter C mineralization (Strickland et al., 2009). In addition, assembled communities of cellulose degraders reveal that the composition of the community has significant impacts on the rate of cellulose degradation (Wohl et al., 2004).

Important steps in understanding soil C cycling dynamics are identifying contributions from specific microbial lineages and investigating the relationship between genetic diversity and community structure with function (O'Donnell et al., 2002). The vast majority of microorganisms continue to resist cultivation in the laboratory, and even when cultivation is achieved, the traits expressed by a microorganism in culture may not be representative of those expressed when in its natural habitat. Stable-isotope probing (SIP) provides a unique opportunity to link microbial identity to activity and has been utilized to expand our knowledge of

biogeochemical processes (Chen, Murrell 2010). The most successful applications of this technique have identified organisms that mediate processes performed by a narrow set of functional guilds such as methanogens (Lu 2005). The technique has been less applicable to the study of soil C cycling because of limitations in resolving power as a result of simultaneous labeling of many different organisms in the community. Additionally, molecular applications - such as terminal restriction fraction length polymorphism (tRFLP), denaturing gradient gel electrophoresis (DGGE), and cloning - that are frequently used in conjunction with SIP provide insufficient resolution of taxon identity and depth of coverage. We have developed an approach called **H**igh **R**esolution-SIP (HR-SIP) that employs a mixture of substrates added to soil at a low concentration relative to soil organic matter pools along with high throughput DNA sequencing of multiple fractions from each nucleic acid isopycnic density gradient. By increasing the number of fractions collected per density gradient, we are able to better assess the magnitude of isotope incorporation (based on density shift) for individual OTUs, which can be leveraged to resolve C use dynamics by microbial community members over time.

During the degradation of plant biomass, a temporal succession occurs in microbial communities in which labile C degradation precedes polymeric C degradation (Hu, Bruggen 1997; Rui et al., 2009). The aim of this study is to track the temporal dynamics of C assimilation through discrete individuals of the soil microbial community to provide greater insight into soil C-cycling. Our experimental approach includes the addition of a soil organic matter (SOM) simulant (a mixture of model C sources and inorganic nutrients common to plant biomass), where a single

C constituent is substituted for its ^{13}C-labeled equivalent, to soil. Parallel incubations of soils amended with this mixture allow us to test how different C substrates flow through discrete taxa within the soil microbial community. In this study we use ^{13}C-xylose and ^{13}C-cellulose as general proxies for labile and polymeric C, respectively. We couple nucleic acid stable isotope probing with high throughput DNA sequencing to identify soil microbial community members responsible for specific C transformations. Amplicon sequencing of 16S rRNA gene fragments from many gradient fractions and multiple gradients make it possible to track C assimilation by hundreds of taxa over time.

2.3 Methods

2.3.1 Soil collection and preparation

Soils were collected from an organic farm in Penn Yan, New York. These soils are Honoeye/Lima, a silty clay loam on calcareous bedrock. Twelve soil cores (5 cm diameter x 10 cm depth) were collected in duplicate from six random sampling locations in a single field by using a slide hammer bulk density sampler (coordinates: (1) N 42° 40.288' W 77° 02.438', (2) N 42° 40.296' W 77° 02.438', (3) N 42° 40.309' W 77° 02.445', (4) N 42° 40.333' W 77° 02.425', (5) N 42° 40.340' W 77° 02.420', (6) N 42° 40.353' W 77° 02.417') on November 21, 2011. Cores were sieved to 2 mm, homogenized, and stored at 4°C (for 1-2 weeks). Carbon and nitrogen content have previously been measured for these soils (Berthrong et al., 2013). Reported values were 12.15 ± 0.78 (s.d.) mg C g^{-1} dry weight (d.w.) soil and 1.16 ± 0.13 (s.d.) mg N g^{-1} d.w. soil.

2.3.2 Cellulose production

Bacterial cellulose (both ^{12}C and ^{13}C) was produced by *Gluconoacetobacter xylinus* grown in Heo and Son (Heo, Son 2002) liquid minimal medium made with 0.1% glucose. Specifically, cellulose was produced in 1 L Erlenmeyer flasks containing 100 mL Heo and Son minimal medium that were inoculated with three colonies of *Gluconoacetobacter xylinus* grown on Heo and Son 0.1% glucose agar plates without inositol at 30°C. Flasks were incubated statically in the dark at 30°C for 2-3 weeks until a thick cellulose pellicule had formed. Cellulose pellicules were collected and autoclaved for 30 min with two volumes 1% Alconox. Cellulose pellicules were rinsed repeatedly with deionized water then purified by dialysis in 1 L deionized water for 12 hrs. Dialysis was repeated 10 times. Pellicules were then dried overnight (60°C), cut into pieces, and ground to 53 μm – 250 μm using 5100 Mixer/Mill (SPEX SamplePrep, Metuchen, NJ) and dry sieved. The particulate size range was selected to be representative of particulate organic matter in soils (Cambardella, Elliott 1992).

The purity of ground cellulose was checked by a biological assay, Benedict's reducing sugars assay, Bradford assay, and isotopic analysis. *E. coli* is not able to use cellulose as a C source but is capable of growth on nutrients available in the Heo and Son medium and on nutrients found in dead bacterial cells. The biological assay consisted of *E. coli* inoculated into 5 mL minimal M9 medium which lacked a C source and was supplemented with either: (1) 0.01% glucose, (2) 2.5 mg purified, ground cellulose, (3) 25 mg purified, ground cellulose, (4) 25 mg purified, ground cellulose and 0.01% glucose. Growth was evaluated by spectrometer (OD_{450}). No

measureable growth was observed with either 2 mg or 25 mg cellulose, indicating absence of contaminating nutrients that can be metabolized by *E. coli*. In addition, the presence of 25 mg cellulose did not inhibit the growth of *E. coli* cultures provided with glucose (relative to control), indicating the absence of compounds in the purified cellulose that may inhibit microbial growth (data not shown).

Purified cellulose was also assayed for residual proteins and sugars using Bradford and Benedict's assays, respectively. Bradford assay was performed as in Bradford (1976). Ground, purified cellulose contained 6.92 μg protein mg cellulose^{-1}. Reducing sugars were not detected in cellulose using Benedict's reducing sugar assay (Benedict 1909) tested at 10 mg cellulose ml^{-1}. Finally, ^{13}C-cellulose had an average 96 ± 5 (s.d.) atom % ^{13}C as determined by isotopic analysis (UCDavis Stable Isotope Facility).

2.3.3 Soil microcosms

An aliquot of soil was dried at 105°C overnight to determine soil moisture content gravimetrically. Microcosms (35 total) were created by adding 10 g soil dry weight (of the sieved soil) to a 250 mL Erlenmeyer flask, capped with a butyl rubber stopper. The headspace was flushed with air every 3 days. Microcosms were pre-incubated at 25°C for 2 weeks until the soil respiration rate (determined by GCMS measurement of head space CO_2) had stabilized. Sieving causes a transient increase in soil respiration rate presumably due to the liberation of fresh labile soil organic matter (Datta et al., 2014). Pre-incubation ensures that this labile organic matter is

consumed and/or stabilized prior to the beginning of the experiment. Respiration rate stabilized after 10 days.

Three treatments of soil microcosms were established. Each treatment received the same composition of amendments, where the only difference is the identity of the C constituent that is substituted for its ^{13}C-labeled equivalent. All treatments received 2 mg cellulose g^{-1} dry weight (d.w.) soil (0.88 mg cellulose-C g^{-1} d.w. soil), lignin (1.2 mg g^{-1} d.w. soil), and an aqueous organic matter simulant (0.12 mL g^{-1} d.w. soil). The organic matter simulant includes a mixture of organic and inorganic nutrients of fresh organic matter and is described below. The aqueous amendment constituted (by mass) 5.3 mg g^{-1} d.w. soil, representative of natural concentrations (Schneckenberger et al., 2008). A total of 34 microcosms were established, 12 replicates for ^{13}C-xylose treatment, 12 for the control treatment (no ^{13}C-isotope), and 10 for ^{13}C-cellulose treatment.

Microcosm amendments are shorthand identified by the following code: "13CXPS" refers to the amendment with ^{13}C-xylose (that is ^{13}**C** **X**ylose **P**lant **S**imulant), "13CCPS" refers to the ^{13}C-cellulose amendment (^{13}**C** **C**ellulose **P**lant **S**imulant), and "12CCPS" refers to the amendment that only contained ^{12}C substrates (i.e. control).

The organic matter simulant was designed based on switch grass biomass composition (Yan et al., 2010; David, Ragauskas 2010) to include (by mass) 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose, with the remaining 13.5% mass composed of amino acids (in-house made replica of Teknova Cat#C0705) and basal salt mixture (Murashige and Skoog,

Sigma M5524). This mixture produced a C:N of 10. The volume of the liquid addition (0.12 mL g^{-1} d.w. soil) was chosen to achieve 50% water holding capacity of the soil. Water holding capacity of 50% was chosen to achieve ~70% water filled pore space in these soils based on soil texture, which is the optimal water content for respiration (Linn, Doran 1984).

Replicate microcosms were incubated in parallel and sampled destructively at days 1 (control and xylose only), 3, 7, 14, and 30 (Figure 2.1). Harvested microcosms were stored at -80°C until nucleic acid processing. An aliquot of microcosm soil for each treatment and time point were isotopically analyzed at Cornell University Stable Isotope Laboratory to determine amount of ^{13}C that remained at each time point.

2.3.4 Nucleic acid extraction

Nucleic acids were extracted from 0.25 g soil using a modified Griffiths procotol (Griffiths et al., 2000). Cell lysis was performed by bead beating for 1 min at 5.5 m s^{-1} in 2 mL tubes containing 0.5 g of 0.1 mm diameter silica/zirconia beads (pretreated at 300°C for 4 hours to remove RNases), 0.5 mL extraction buffer (240 mM phosphate buffer and 0.5% N-lauryl sarcosine), and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1) for 1 min at 5.5 m s^{-1}. After lysis, 85 µL 5 M NaCl and 60 µL 10% hexadecyltriammonium bromide (CTAB)/0.7 M NaCl were added, vortexed, chilled for 1 min on ice, and centrifuged at 16,000 x g for 5 min at 4°C. The aqueous layer was transferred to a new tube and reserved on ice. To increase DNA recovery, the soil pellet was back extracted with 85 µL 5 M NaCl and 0.5 mL extraction buffer.

The aqueous extract was washed with 0.5 mL chloroform:isoamyl alcohol (24:1). Nucleic acids were precipitated by addition of 2 volumes polyethylene glycol solution (30% PEG 8000, 1.6 M NaCl) and a 2 hr incubation on ice, followed by centrifugation at 16,000 x g, 4°C for 30 min. The supernatant was discarded and nucleic acid pellet was washed with 1 mL ice cold 70% EtOH. The nucleic acid pellet was air dried, resuspended in 50 µL TE and stored at -20°C.

DNA was size selected (>4 kb) to prepare nucleic acid extracts for isopycnic centrifugation as previously described (Buckley et al., 2007). Briefly, DNA was size separated using 1% low melt agarose gel, bands >4 kb were excised then purified from the gel using β-agarase I enzyme extraction per manufacturers protocol (New England Biolab, M0392S). Final resuspension of DNA pellet was in 50 µL TE.

2.3.5 Isopycnic centrifugation and fractionation

For each time point in the series, isopycnic gradients were setup using a modified protocol from Neufeld et al.(2007) for a total of five ^{12}C-control, five ^{13}C-xylose, and four ^{13}C-cellulose microcosms (Figure 2.1). A cesium chloride (CsCl) density gradient solution of an average density 1.69 g mL^{-1} was used to separate ^{13}C-labeled and unlabeled (^{12}C) DNA. The gradient buffer (pH 8.0) used for the density gradient solution was composed of 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl. Each centrifuge tube was loaded with the CsCl density gradient solution and approximately 5 µg of DNA, then centrifuged on a Beckman Coulter Optima™ MAX-E ultracentrifuge using a TLA-110 fixed-angle rotor for 66 h at 55,000 rpm and room temperature (RT).

Fractions of ~100 µL were collected from below the centrifugation tube by displacing the DNA-CsCl-gradient buffer solution in the tube with water using a syringe pump at a flow rate of 3.3 µL s^{-1} (Manefield et al., 2002) into Acroprep™ 96 filter plate (Pall Life Sciences, PN: 5035,). The refractive index (R_i) of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described (Buckley et al., 2007) to measure a volume of 5 µL. The R_i was corrected to account for the R_i of the gradient buffer using the equation $[R_{i\ corrected}] = [R_{i\ observed}] - ([R_{i\ buffer}] - 1.3333)$. Then the buoyant density was calculated from the $[R_{i\ corrected}]$ using the equation $\rho = a\eta - b$, where ρ is the density of the CsCl (g ml^{-1}), η is the $[R_{i\ corrected}]$, and *a* and *b* are coefficient values of 10.9276 and 13.593, respectively, for CsCl at 20°C (Birnie 1978).

Each well in the Acroprep filter plate contained a single fraction from a CsCl gradient. The collected DNA fractions were purified by washing the Acroprep filter wells five times with 200 µL TE followed by a 10 min centrifugation at 500 x g. Finally, 50 µL TE was added to each well then resuspended DNA was pipetted off the filter into a new microfuge tube.

The number of 16S rRNA genes in each fraction were quantitated by qPCR (Bio-Rad C1000/CFX96 thermocycler) as described previously (Berthrong et al., 2013) using 12.5 µL QuantiFast SYBR green PCR master mix (Qiagen, Valencia, CA; 204056), 1.25 µL 10 µM 515F primer (5'-GTGCCAGCMGCCGCGGTAA -3'), 1.25 µL 10 µM 806R primer (5'- GGACTACHVGGGTWTCTAAT -3'), and 1:100 dilution of DNA template. To estimate the abundances of rRNA gene copies, we used standard curves from 10-fold serial dilutions of 16S rRNA gene amplicons generated

from *Klebsiella pneumoniae* using the same primers. The thermocycler conditions for amplification were 95°C for 5 min followed by 40 cycles of 95°C for 10 s, 53°C for 30 s, and 72°C for 30 s, followed by a final elongation at 72°C for 5 min.

2.3.6 DNA sequencing

For every gradient, 20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL^{-1} (Figure 2.1). A total of 14 gradients (277 fractions) and their corresponding bulk DNA extraction (after *β*-agarase size selection) were amplified for sequencing. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes (Hamady et al., 2008), a 2 bp linker (5'-CA-3'), and 806R primer for reverse primer (BA806R); and 454-specific adapter A, a 2 bp linker (5'-TC-3'), and 515F primer for forward primer (BA515F). Each fraction was PCR amplified in triplicate using 0.25 µL 5 U $µl^{-1}$ AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 2.5 µL 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5 µL 25 mM $MgCl_2$, 4 µL 5 mM dNTP, 1.25 µL 10 mg mL^{-1} BSA, 0.5 µL 10 µM BA515F, 1 µL 5 µM BA806R, 3 µL H_2O, and 10 µL 1:30 DNA template. The same thermocycler conditions were used as described above except 22 cycles were used instead of 40. Amplification products were checked by 1% agarose gel electrophoresis. Samples were normalized either using Quant-IT pico green quantification (Life Technologies, Grand Island, NY; P7589) and manual calculation or by SequalPrep™ normalization plates (Invitrogen, Carlsbad, CA; A10510), then pooled in equimolar concentrations. A final purification of pooled DNA was performed via gel extraction from a 1% agarose gel using Wizard SV gel and PCR clean-up system (Promega, Madison, WI; A9281) per manufacturer's protocol.

Amplicons were sequenced on Roche 454 FLX system using titanium chemistry at Selah Genomics (formerly EnGenCore, Columbia, SC).

2.3.7 Post-sequencing analysis

Sequence quality control

Sequences were initially screened by maximum expected errors at a specific read length threshold (Edgar 2013), which has been shown to be as effective as denoising with respect to removing pyrosequencing errors. Specifically, reads were first truncated to 250 nucleotides (nt) (all reads shorter than 250nt were discarded) and any read that exceeded a maximum expected error threshold of 0.5 was removed. After truncation and max-expected error trimming, 87% of original reads remained. Forward primer and barcode was then removed from the high quality, truncated reads. Remaining reads were taxonomically annotated using the "UClust" taxonomic annotation framework in the QIIME software package (Edgar 2010; Caporaso et al., 2010) with cluster seeds from Silva SSU rRNA database (Pruesse et al., 2007) 97% sequence identity OTUs as reference (release 111). Reads annotated as "Chloroplast", "Eukaryota", "Archaea", "Unassigned" or "mitochondria" were removed from the dataset. Finally, reads were aligned to the Silva reference alignment provided by the Mothur software package (Schloss et al., 2009) using the Mothur NAST aligner (DeSantis et al., 2006). All reads that did not align to the expected amplicon region of the SSU rRNA gene were discarded. Quality control parameters removed 617,795 reads of 1,720,480 raw reads.

Sequence clustering

Sequences were distributed into OTUs using the UParse methodology (Edgar 2013). Specifically, OTU centroids (i.e. seeds) were identified using USearch on non-redundant reads sorted by count. The sequence identity threshold for establishing a new OTU centroid was 97%. With USearch/UParse, potential chimeras are identified during OTU centroid selection and are not allowed to become cluster centroids effectively removing chimeras from the read pool. All quality controlled reads were then mapped to cluster centroids at an identity threshold of 97% again using USearch. 97% of quality control reads could be mapped to centroids. Unmapped reads do not count towards sample counts and are removed from downstream analyses. The USearch software version for cluster generation was 7.0.1090.

Phylogenetic analysis

Alignment of OTU centroid SSU rRNA genes was done with SSU-Align, which is based on Infernal (Nawrocki et al., 2009; Nawrocki, Eddy 2013). Columns in the alignment that were not included in the SSU-Align covariance models or were aligned with poor confidence (less than 95% of characters in a position had posterior probability alignment scores of at least 95%) were masked for phylogenetic reconstruction. Additionally, the alignment was trimmed to coordinates such that all sequences in the alignment began and ended at the same positions. FastTree (Price et al., 2009) was used to reconstruct the phylogeny.

Community and sequence analysis

Nonmetric Multidimensional Scaling (NMDS) uses a distance (or dissimilarity) matrix to find the least stressed relationship between samples in a low dimensional space. Specifically, weighted unifrac (Lozupone, Knight 2005) distances were used for NMDS analyses. The Phyloseq (McMurdie, Holmes 2013) wrapper for Vegan (Dixon 2003) (both R packages) was used to compute sample values along the axes. The ordinations presented here are graphical representations of the sample relationships as determined by NMDS analysis. GGplot2 (Wickham 2009) was used to display sample points in two-dimensional space. In general, samples in close proximity have more similar microbial composition than samples spaced further away. Adonis tests (Anderson 2001) were done with 1000 permutations to compare community compositions.

Identifying OTUs that incorporated ^{13}C into their DNA

DNA-SIP is a culture-independent approach towards defining identity-function connections in microbial communities (Buckley 2011; Neufeld et al., 2007; Radajewski et al., 2003). Microbes are identified on the basis of isotope assimilation into DNA. As the buoyant density (BD) of a macromolecule is dependent on many factors (e.g. G+C-content in nucleic acids (Youngblut, Buckley 2014)) in addition to stable isotope incorporation, labeled nucleic acids from one microbial population may have the same BD as unlabeled nucleic acids from another. Therefore, it is imperative to compare results of isotopic labeling to results obtained with unlabeled controls where everything mimics the experimental conditions except that

unlabeled substrates are used. By contrasting heavy gradient fractions from isotopically labeled samples relative to corresponding fractions from controls, the identities of microbes with labeled nucleic acids can be determined (Figure 2.2).

We used a RNA-Seq differential expression statistical framework (Love et al., 2014) to find OTUs enriched in heavy fractions of labeled gradients relative to corresponding density fractions in control gradients (Figure 2.2)(for review of RNA-Seq differential expression statistics applied to microbiome OTU count data see (McMurdie, Holmes 2014)). We use the term "differential abundance" coined by McMurdie *et al.* (2014) to denote OTUs that have different proportion means across sample classes (in this case the only sample class is labeled:control). CsCl gradient fractions were categorized as "heavy" or "light". The heavy category denotes fractions with density values between 1.7125-1.755 g mL^{-1}. Since we are only interested in enriched OTUs (labeled versus control), we used a one-sided Wald-test for differential abundance (the null hypothesis is the labeled:control proportion mean ratio for an OTU is less than a selected threshold). P-values were corrected with the Benjamini and Hochberg method (Benjamini, Hochberg 1997).

We selected a threshold of 0.75 (or a labeled:control proportion mean ratio of 1.68). Only OTUs present in at least 60% of the density fraction libraries (within the 1.7125-1.755 g mL^{-1} density window) were evaluated with DESeq2. DESeq2 was used to calculate the moderated $\log_2$ fold change of labeled:control proportion mean ratios and corresponding standard errors for the Wald test. Mean ratio moderation allows for reliable ratio ranking such that high variance and likely statistically insignificant mean ratios are appropriately shrunk and subsequently ranked lower

than they would be as raw ratios. Those OTUs that exhibit a statistically significant proportional increase, and pass a false discovery rate of 0.1, in heavy fractions from ^{13}C-labeled samples relative to corresponding controls have increased significantly in buoyant density in response to ^{13}C treatment. OTUs that significantly assimilated ^{13}C into their DNA were identified by BLAST searches that were done with the "blastn" program from BLAST+ toolkit (Camacho et al., 2009) version 2.2.29+. Default parameters were always employed and the BioPython (Cock et al., 2009) BLAST+ wrapper was used to invoke the blastn program. Pandas (McKinney 2012) and dplyr (Wickham, Francois 2014) were used to parse and transform BLAST output tables.

Estimated rrn gene copy number

The number of ribosomal (*rrn*) gene copies per genome reflects ecological strategies in bacteria (Klappenbach et al., 2000). The more *rrn* gene copies a microorganism has, the more quickly it can replicate (i.e. growth rate, Yano et al., 2013). Using the method described in Kembel *et al.* (2012) we estimated the *rrn* gene copy number for ^{13}C-xylose and ^{13}C-cellulose responders. In brief, the aligned environmental sequences (described above) are placed on a reference phylogeny containing taxa with known copy numbers using pplacer (Matsen et al., 2010). The new root node of the phylogeny is then used to estimate copy number and branch length connecting the root and the novel taxon are used to estimate uncertainty (Kembel et al., 2012).

2.4 Results

We sequenced SSU rRNA gene amplicons from a total of 277 gradient fractions from 14 CsCl gradients and 12 bulk microcosm DNA samples. The SSU rRNA gene data set contained 1,102,685 total sequences. The average number of sequences per sample was 3,816 (s.d. 3,629) and 265 samples had over 1,000 sequences. We sequenced SSU rRNA gene amplicons from ~20 fractions per gradient. The average density between fractions was 0.0040 g mL^{-1}. The sequencing effort recovered a total of 5,940 OTUs. We observed 33 unique phylum and 340 unique genus annotations.

2.4.1 Soil microbial community changes with time in response to C amendment

OTUs in the bulk samples represented only 2,943 of the 5,940 total OTUs detected. Changes in the bulk soil microbial community structure correlated significantly with incubation time (Figure 2.3, Figure 2.4; Adonis, p-value: 0.023, R^2: 0.63). The identity of the ^{13}C-labeled substrate had no significant effect on the overall bulk microbial community structure (Figure 2.3; Adonis, p-value: 0.35, R^2: 0.21). Twenty-nine OTUs significantly changed in relative abundance in the bulk microbial community with time ("BH" adjusted p-value < 0.10; (Y Benjamini 1995)) (Figure 2.5). OTUs that significantly increased in relative abundance with time included OTUs in *Verrucomicrobia*, *Proteobacteria*, *Planctomycetes*, *Cyanobacteria*, *Chloroflexi* and *Acidobacteria*. OTUs that significantly decreased in relative abundance with time included OTUs in *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Figure 2.5). *Proteobacteria* was the only phylum that had OTUs that

both increased and decreased significantly with time. If sequences were grouped by class level, only four classes significantly changed in abundance, *Bacilli* (decreased), *Flavobacteria* (decreased), *Gammaproteobacteria* (decreased) and *Herpetosiphonales* (increased) (Figure 2.6). Of the 29 OTUs that changed significantly in relative abundance with time, 14 were subsequently classified as responding to ^{13}C-labeled substrates (see below).

2.4.2 Dynamics of ^{13}C-xylose and ^{13}C-cellulose assimilation

High-resolution SIP (HR-SIP) revealed the dynamics of xylose and cellulose metabolism in soil. Soils having 12.15 ± 0.78 (s.d.) mg C g^{-1} (d.w.) received a total amendment of 2.99 mg C g^{-1} soil (d.w.) which included 0.42 mg xylose-C (i.e. 3.4 % of total soil C) and 0.88 mg cellulose-C g^{-1} soil (d.w.) (i.e. 7.2% of total soil C). Assimilation of ^{13}C from xylose began at day 1, diminishing over the 30 day incubation, whereas assimilation of ^{13}C from cellulose began two weeks after amendment additions (Figure 2.7).

'Heavy' fraction amplicon pools from samples that received ^{13}C-xylose diverged from corresponding controls on days 1 through 7 (Figure 2.7). This corresponded to approximately 63% of ^{13}C from xylose lost from soil in the first 7 days (Table 2.1). Furthermore, amplicon pool composition varied across these days indicating dynamic changes in ^{13}C-xylose assimilation with time (Figure 2.7). At days 14 and 30 heavy fractions from ^{13}C-xylose labeled samples were no longer differentiated from corresponding controls indicating a loss of ^{13}C detection in DNA and during this time only an additional 6% more of ^{13}C from xylose was respired

from soil (Figure 2.7, Table 2.1). The decline in ^{13}C-labelling of DNA is likely due to isotopic dilution resulting from assimilation of unlabeled C and/or due to cell turnover resulting from mortality. At the end of the 30 day incubation 30% of the ^{13}C from added xylose remained in the soils.

^{13}C-cellulose 'heavy' fraction amplicon pools diverged from corresponding controls on days 14 and 30 (Figure 2.7). An average 16% of the ^{13}C-cellulose added was respired within the first 7 days, 38% by day 14, and 60% by day 30 (Table 2.1). At the end of the experiment 40% of the original ^{13}C from cellulose remained in the soil.

2.4.3 OTUs that assimilated ^{13}C into their DNA

Isotope incorporation by an OTU is revealed by enrichment of the OTU in heavy fractions from ^{13}C-labeled samples relative to corresponding heavy fractions from unlabeled controls (Figure 2.2). We identify OTUs that are enriched in the heavy fractions of ^{13}C-labeled samples relative to controls as 'responders'. We detected 49 and 63 unique OTUs that responded to ^{13}C-xylose and ^{13}C-cellulose, respectively (Figure 2.8, Figure 2.9).

Xylose OTUs

Xylose responder abundances, summed at the phylum level, decreased over time for *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* (Figure 2.10), although *Proteobacteria* spiked at day 14. These were the same trends observed in the bulk community (discussed above, Figure 2.5). At day 1, 84% of ^{13}C-xylose responsive OTUs belonged to *Firmicutes*, 11% to *Proteobacteria,* and 5% to

Bacteroidetes. *Firmicutes* responders decreased from 16 OTUs at day 1 to one OTU at day 3 while *Bacteroidetes* responders increased from one OTU at day 1 to 12 OTUs at day 3. The remaining day 3 responders are members of the *Proteobacteria* (26%) and the *Verrucomicrobia* (5%). Day 7 responders were 53% *Actinobacteria*, 40% *Proteobacteria*, and 7% *Firmicutes*. The identities of ^{13}C-xylose responders change with time; the numerically dominant ^{13}C-xylose responder phylum shifts from *Firmicutes* to *Bacteroidetes* and then to *Actinobacteria* across days 1, 3 and 7 (Figure 2.11, Figure 2.12).

All of the ^{13}C-xylose responders in the *Firmicutes* phylum are closely related (at least 99% sequence identity) to cultured isolates from genera that are known to form endospores (Table 2.2). Each *Firmicutes* ^{13}C-xylose responder is closely related to isolates annotated as members of *Bacillus*, *Paenibacillus* or *Lysinibacillus*. *Bacteroidetes.* ^{13}C-xylose responders are predominantly closely related to *Flavobacterium* species (5 of 8 total responders)(Table 2.2). Six of the eight *Actinobacteria* ^{13}C-xylose responders are in the *Micrococcales* order. One ^{13}C-xylose responding *Actinobacteria* OTU shares 100% sequence identity with *Agromyces ramosus* (Table 2.2).

Cellulose OTUs

Cellulose responder abundances summed at phylum level generally increased over time in the bulk microbial community (Figure 2.10). Only 2 (*Cellvibrio* and *Sandaracinaceae*) and 5 (*Cellvibrio*, a *Verrucomicrobia* OTU and three *Chloroflexi*) OTUs had incorporated ^{13}C from cellulose at days 3 and 7, respectively.

At days 14 and 30, 42 and 39 OTUs incorporated ^{13}C from cellulose into biomass. The dominant OTUs assimilating ^{13}C-cellulose on days 14 and 30 belonged to *Proteobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Chloroflexi* (Figure 2.11).

Proteobacteria represent 46% of all ^{13}C-cellulose responding OTUs identified. *Cellvibrio*, an established cellulose degrader, accounted for 3% of all proteobacterial ^{13}C-cellulose responding OTUs detected. One ^{13}C-cellulose responder is annotated as "cyanobacteria" (Table 2.3). The cyanobacteria phylum annotation is misleading, as the OTU is not closely related to any oxygenic phototrophs. It is also not related to any cultured type strains. However, it most closely matches (96%) *Vampirovibrio*. Recent analyses have reclassified *Vampirovibrio* from *Deltaproteobacteria* to the *Melainabacteria* class in the Cyanobacteria phylum (Hugenholtz et al., 2015).

Other notable ^{13}C-cellulose responders include a *Bacteroidetes* OTU that shares high sequence identity (99%) to *Sporocytophaga myxococcoides* a known cellulose degrader (Vance et al., 1980), and three *Actinobacteria* OTUs that share complete sequence identity (100%) with isolates. One of the three *Actinobacteria* ^{13}C-cellulose responders is in *Streptomyces*, a genus known to possess cellulose degraders, while the other two share high sequence identity to cultured isolates *Allokutzneriz albata* (Labeda, Kroppenstedt 2008; Tomita et al., 1993) and *Lentzea waywayandensis* (Labeda, Lyons 1989; Labeda et al., 2001); neither isolate decomposes cellulose in culture. Nine *Planctomycetes* OTUs responded to ^{13}C-cellulose but none are within described genera (Table 2.3, Figure 2.13).

Xylose and cellulose shared responders

There were 8 shared responders among all unique responders identified in both the xylose and cellulose treatments (n = 104); several *Proteobacteria* (*Xanthomonadales*, two *Rhizobiales*, *Myxococcales*, *Burkholderiales*, *Cellvibrio*), a *Planctomycetes* (*Planctomyces*), and a *Verrucomicrobia* (*Spartobacteria*). Four of the shared responders corresponded in time between the two treatments; *Myxococcales* (day 3), *Cellvibrio* (day 3), *Planctomyces* (day 14), and *Spartobacteria* (day 14).

2.4.4 Ecological characteristics of responders

Xylose responders are more abundant members of the soil community than cellulose responders

^{13}C-xylose responders are generally more abundant members (0.002 ± 0.004 s.d.) of the microbial community based on ranked relative abundance in bulk DNA SSU rRNA gene content than ^{13}C-cellulose responders (mean relative abundance 0.0007 ± 0.002 s.d.)(Figure 2.14; ANOVA, p-value: 0.00028). However, abundant and rare OTU responders are found for both ^{13}C-xylose and ^{13}C-cellulose (Figure 2.14). For instance, *Delftia*, a ^{13}C-cellulose responder is fairly abundant in the bulk samples ("OTU.5", Table 2.3). OTU.5 was on average the 13th most abundant OTU in bulk samples. On the other hand, a ^{13}C-xylose responder ("OTU.1040", Table 2.2) has a mean relative abundance in bulk samples of 3.57×10^{-05}. Notably, two ^{13}C-cellulose responders were not found in any bulk samples ("OTU.862" and "OTU.1312", Table 2.3). Of the 10 most abundant responders, 8 are ^{13}C-xylose responders and 6 of these 8 are consistently among the 10 most abundant OTUs in bulk samples.

Cellulose degraders exhibit higher substrate preference than xylose utilizers

We measured the change in the center of mass (deltaCM) from an OTU's density profile between corresponding control and labeled gradients as a metric for ^{13}C assimilation (Figure 2.2). The center of mass (CM) of DNA increases as its ratio of ^{13}C to ^{12}C increases. Cellulose responders exhibited a greater deltaCM than xylose responders in response to isotope incorporation (Figure 2.14; p-value: 1.8610×10^{-06}). ^{13}C-cellulose responders shifted on average 0.0163 g mL^{-1} (s.d. 0.0094) whereas xylose responders shifted on average 0.0097 (s.d. 0.0094). CM shifts, however, should not be evaluated on an individual OTU basis as a small number of CM shifts are observed for each OTU and the variance of the CM shift metric at the level of individual OTUs is unknown. It is therefore more informative to compare CM shifts among substrate responder groups. It should also be noted that there was overlap in observed density shifts between ^{13}C-cellulose and ^{13}C-xylose responder groups, suggesting that although cellulose degraders generally have greater substrate preference than xylose responders, each responder group exhibits a range of substrate preference (Figure 2.14). Although we observed a succession of ^{13}C-xylose responders (Figure 2.11, Figure 2.12), there was no discernible difference in deltaCM (i.e. substrate preference) between ^{13}C-xylose responders at days 1, 3 or 7.

Estimated rrn gene copy number in substrate responder groups

^{13}C-xylose responder estimated *rrn* gene copy number is inversely related to time of first response (Figure 2.15; ANOVA, p-value: 2.02×10^{-15}). Xylose responder OTUs had a mean *rrn* gene copy number of 9.6 ± 1.7 (s.d.) at day 1, 5.2 ± 2.1 (s.d.) at

day 3, and 3.7 ± 2.4 (s.d.) at day 7. In other words, xylose responsive OTUs that first responded at earlier time points had more *rrn* gene copy numbers than OTUs that first responded at later time points (Figure 2.15). *rrn* copy number estimation is a recent advance in microbiome science (Kembel et al., 2012) although the relationship of *rrn* copy number per genome with ecological strategy is well established (Klappenbach et al., 2000). Microorganisms with a high *rrn* copy number tend to be fast growers specialized to take advantage of boom-bust environments whereas microorganisms with low *rrn* copy number favor slower growth under lower and more consistent nutrient input (Klappenbach et al., 2000). At the beginning of our incubation, OTUs with estimated high *rrn* copy number or "fast-growers" assimilate xylose into biomass and, with time, slower growers (lower *rrn* copy number) began to incorporate ^{13}C from xylose into DNA. Further, ^{13}C-xylose responders have more estimated rRNA operon copy numbers per genome than ^{13}C-cellulose responders (Figure 2.15; p-value: 1.878×10^{-09}) suggesting xylose respiring microbes are generally faster growers than cellulose degraders. This trend is supported by the measured abundances of responder OTUs over time (Figure 2.10) such as *Firmicutes* (high *rrn* copy number, fast response) and *Verrucomicrobia* (low *rrn* copy number, slow response).

2.5 Discussion

Historically only nine genera were recognized as important soil microbes based on culturing methodologies: *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Micromonospora*, *Nocardia*, *Pseudomonas*, and

Streptomyces ((Alexander 1977) as reviewed by (Janssen 2006)). More recent culture-independent surveys of soil microbial diversity revealed soils can harbor 5,000 OTUs per half gram of soil (Schloss, Handelsman 2006) and that cultured isolates did not represent *in situ* numerically abundant genera. We recovered 5,940 OTUs in this study. Although culturing techniques can produce isolates from diverse soil phylogenetic lineages (Janssen et al., 2002), numerically dominant soil microorganisms are still uncultured and we know little of their ecophysiology (Janssen 2006). In contrast, DNA-SIP has the potential to characterize functional roles for thousands of phylotypes in a single experiment. Using HR-SIP, we found 104 OTUs in an agricultural soil that can incorporate C from xylose and/or cellulose into DNA. Included in the ^{13}C-xylose and ^{13}C-cellulose responsive OTUs were members of numerically dominant yet functionally uncharacterized soil phylogenetic groups such as *Verrucomicrobia*, *Planctomycetes* and *Chloroflexi*.

2.5.1 Cellulose responders

Cellvibrio was one of the first identified cellulose degrading bacteria and was originally described by Winogradsky in 1929, who named it for its cellulose degrading abilities (Boone 2001). All ^{13}C-cellulose responding *Proteobacteria* share high sequence identity with 16S rRNA genes from sequenced cultured isolates (Table 2.3), with few exceptions. Some *Proteobacteria* responders share high sequence identity with isolates in genera known to possess cellulose degraders including *Rhizobium*, *Devosia*, *Stenotrophomonas* and *Cellvibrio*. One *Proteobacteria* OTU shares high sequence identity (100%) with a *Brevundimonas* cultured isolate.

Brevundimonas has not previously been identified as a cellulose degrader, but has been shown to degrade cellouronic acid, an oxidized form of cellulose (Tavernier et al., 2008).

Verrucomicrobia, a cosmopolitan soil phylum often found in high abundance (Fierer et al., 2013), are hypothesized to degrade polysaccharides in many environments (Fierer et al., 2013; Herlemann et al., 2013; Chin et al., 1999). *Verrucomicrobia* comprised 16% of the total ^{13}C-cellulose responder OTUs detected. 40% of *Verrucomicrobia* ^{13}C-cellulose responders belong to the uncultured "FukuN18" family originally identified in freshwater lakes (Parveen et al., 2013). The strongest *Verrucomicrobia* responder OTU to ^{13}C-cellulose shared high sequence identity to *Luteolibacter* sp. (97%), an isolate from Norway tundra soil (Jiang et al., 2011); although, growth on cellulose was not assessed for this isolate. Only one other ^{13}C-cellulose responding verrucomicrobium shared high DNA sequence identity with an isolate, "OTU.638" (Table 2.3) with *Roseimicrobium gellanilyticum* (100% sequence identity), which has been shown to grow on soluble cellulose (Otsuka et al., 2012). The remaining ^{13}C-cellulose *Verrucomicrobia* responders have no cultivated relatives (Table 2.3) illustrating how little is known about this phylum.

Chloroflexi abundance increased significantly in the bulk community over time (Figure 2.5, Figure 2.6). *Chloroflexi* are metabolically diverse ranging from anoxygenic phototrophy to organohalide respiration (Hug et al., 2013). Recent studies have focused on *Chloroflexi* roles in C cycling (Hug et al., 2013; Goldfarb et al., 2011; Cole et al., 2013) and several *Chloroflexi* are proposed to utilize cellulose

(Goldfarb et al., 2011; Cole et al., 2013; Hug et al., 2013). Four closely related OTUs in an undescribed *Chloroflexi* lineage (Table 2.3) responded to ^{13}C-cellulose (Figure 2.13). One additional OTU also from a poorly characterized *Chloroflexi* lineage responded to ^{13}C-cellulose (Figure 2.13).

Vampirovibrio is the closest match for one cellulose responder, OTU.120. *Vampirovibrio* has recently been proposed to belong to the *Melainabacteria* class in Cyanobacteria (Hugenholtz et al., 2015). *Melainabacteria*, unlike its Cyanobacteria siblings, does not possess any known phototrophs, and has recently been proposed to constitute its own phylum, "*Melainabacteria*" (Rienzi et al., 2013), although, the phylogenetic position of "*Melainabacteria*" is debated (Soo et al., 2014). The catalog of metabolic capabilities associated within cyanobacteria are expanding (Rienzi et al., 2013; Soo et al., 2014). Our findings provide evidence for cellulose degradation within a lineage closely related to but apart from oxygenic phototrophs. Notably, polysaccharide degradation is suggested by an analysis of a *Melainabacteria* genome (Rienzi et al., 2013), but *Vampirovibrio* are known for their predatory characteristics (Coder, Goff 1986). It is unknown if this cellulose responder is acting as a predator of cellulose degraders or directly degrading cellulose.

Although we highlight many ^{13}C-cellulose responders that share high sequence identity with described genera, most ^{13}C-cellulose responders uncovered in this experiment have no cultured isolates (Table 2.3).

2.5.2 Microbial response to organic matter amendment

Similar to other studies on the degradation of C compound mixtures in soil (Semenov et al., 2012; Lee et al., 2011; Bernard et al., 2007), the addition of an organic matter mixture in this study caused a successional pattern of microbial community structure during decomposition with time. We propose that C added to soil microcosms in this experiment took the following path through the microbial food web (Figure 2.16): First, labile soluble C such as xylose was assimilated by fast-growing opportunistic *Firmicutes* spore formers, as seen with ^{13}C-labeled rice callus previously (Lee et al., 2011). Thereafter, ^{13}C was assimilated by a succession of slower growing phylotypes belonging to *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. The delayed response of these bacteria could result from uncoupled growth (Blazewicz et al., 2013), use of secondary products of metabolism or they were predatory (e.g. *Agromyces*) or saprotrophic bacteria. C from polymeric substrates, such as cellulose, entered the bacterial community after 14 days. Well known cellulose degrading bacteria such as *Cellvibrio* degraded cellulose but uncharacterized lineages in the *Chloroflexi*, *Planctomycetes* and *Verrucomicrobia*, specifically the *Spartobacteria*, were also significant contributors to cellulose decomposition.

2.5.3 Ecological strategies of soil microorganisms participating in the decomposition of organic matter

We assessed the ecological characteristics of soil microorganisms *in situ* as a function of ^{13}C-label response (xylose/cellulose), deltaCM resulting from ^{13}C-

assimilation, rank abundance within the community, change in rank abundance over time in response to substrate amendment, and estimation of *rrn* gene copy number each OTU. Generally, *rrn* gene copy number correlates positively with growth rate (Klappenbach et al., 2000) and deltaCM is indicative of substrate preference (see results).

Ecological metrics suggest ^{13}C-cellulose responders grow slower (Figure 2.15) in response to C amendment, have greater substrate preference (i.e. higher deltaCM, Figure 2.14), and are less abundant in the bulk microbial community than ^{13}C-xylose responders (Figure 2.14). However, the higher abundance of xylose responders in the bulk microbial community may also be in part due to the high *rrn* gene copy number per genome resulting in inflated relative abundance. Xylose responders may be more varied in their ecological strategies because some responders did not primarily assimilate xylose (lower deltaCM). Responders with a lower deltaCM may become labeled via simultaneous, multiple substrate use (including unlabeled C sources), predatory interactions, and/or are saprophytes. For instance, the xylose responder *Agromyces ramosus* is a known predatory bacterium but is not dependent on a host for growth in culture (Casida 1983).

We infer that ^{13}C-xylose responders are generalists because they have a lower deltaCM than cellulose responders. ^{13}C-cellulose responders are heavily labeled (higher deltaCM), suggesting that cellulose is their main source of C, a response consistent with a specialist lifestyle. Alternatively, the magnitude of deltaCM (low or high) may be an artifact of proximity to the labeled substrate. ^{13}C-xylose responders vary in growth rate and while generally higher abundance than

^{13}C-cellulose responders can also be low abundance microorganisms. It's not clear whether the observed activity succession from *Firmicutes* to *Bacteroidetes* and finally *Actinobacteria* in response to ^{13}C-xylose addition marks a food chain or functional groups tuned to different resource concentrations or both. Notably, each temporally defined response group clustered phylogenetically suggesting a uniform ecological strategy (Figure 2.9, Figure 2.13).

Relatives of all shared responders (except the *Verrucomicrobia*) have been shown to exhibit motility (although this is not demonstrated in this study), which facilitates access to attractive, non-diffusible substrates (Lueders et al., 2006; Wen et al., 1999; Robinson et al., 1992; Mergaert et al., 2003; Fuerst 2013). Additionally, many of these lineages, such as *Xanthomonadales* and *Myxococcales* (which we detected in this study), have been noted as micropredators (Lueders et al., 2006). The presence of taxa with predatory potential as responders for both xylose and cellulose may suggest ^{13}C-labeling resulting from predation of xylose and cellulose degraders rather than direct assimilation of C from xylose and cellulose. If these trophic interactions are real then they may be of importance to soil C turnover models. On the other hand, if the shared responders are not acting as micropredators, the motility of the shared responders for the two treatments may be an advantageous ecological strategy for accessing C substrates.

How – or if – phylogenetic composition affects SOM dynamics is an open question. Phylogenetic composition could affect SOM dynamics if SOM transformations were not functionally equivalent traits and if biology is rate limiting for key C transformations. Alternatively, even with functional redundancy, resource

allocation at the cell level can influence SOM fate (Kindler et al., 2006). It is likely that the ability to carry out soil C transformations are redundant within and between soil microbial communities and that in the mineral soil abiotic factors are rate limiting (Sollins et al., 1996; Kalbitz et al., 2000). Therefore, phylogenetic composition in mineral soil likely influences soil C fate as opposed to dynamics. We demonstrate a phylogenetically coherent response to soluble C additions – for instance, most of the initial response to xylose can be attributed to aerobic spore formers. Assuming cellular resource allocation is consistent with phylogeny, it follows then that phylogenetic composition can significantly influence SOM fate. Polymeric C, on the other hand, did not show the same phylogenetic coherence as soluble C decomposition in this study. This suggests that resource allocation among cellulose degraders would not have a single phylogenetic signal and the fate of polymeric C would not be tied to phylogenetic composition. Though cellulose degraders as a whole likely allocate C differently than labile C degraders.

Within each phylum we observed substrate utilization of xylose or cellulose at the clade or single taxon level with each exhibiting a unique pattern of ^{13}C-assimilation over time. An analysis of the relationships between soil properties and the relative abundances of bacterial phyla in 71 soil samples suggested that all taxa within a phylum are unlikely to share ecological characteristics (Fierer et al., 2007) and possibly even within a species population as seen with marine *Vibrionaceae* (Preheim et al., 2011; Hunt et al., 2008) and reviewed by (Choudoir et al., 2012). Habitat traits of coastal *Vibrionaceae* isolates were mapped onto microbial phylogeny revealing discrete ecological populations based on seasonal occurrence

and particulate size fractionation (Preheim et al., 2011; Hunt et al., 2008). Still, ecological cohesiveness at broad taxonomic groups is debated (Schimel, Schaeffer 2012). The data presented here support that specific functional attributes can be shared among diverse, yet distinct, taxa while closely related taxa may have very different physiologies (Fierer et al., 2012; Philippot et al., 2010). Our results suggest that while substrate utilization is not defined at the level of OTU, it is also not defined at the phylum or even family level (Figure 2.9, Figure 2.13). Our measurements did define differences between and within substrate responder groups suggesting there are ecological strategy sub-groups within larger groups defined by an affinity for a particular substrate (Figure 2.9, Figure 2.13). Hence, compositional changes could occur at different phylogenetic scales in response to environmental perturbation.

Xylose and/or cellulose responders spanned multiple phyla, each revealing a high diversity of bacteria able to utilize these substrates. The high taxonomic diversity may enable substrate metabolism under a broad range of environmental conditions (Goldfarb et al., 2011). Other studies of microbial communities have observed a positive correlation with taxonomic or phylogenetic diversity and functional diversity (Fierer et al., 2012; Fierer et al., 2013; Philippot et al., 2010; Tringe 2005; Gilbert et al., 2010; Bryant et al., 2012).

2.5.4 Conclusion

In this experiment microbes from uncharacterized yet ubiquitous, and often abundant, soil lineages participated in cellulose decomposition. Cellulose C

degraders included members of the *Verrucomicrobia* (*Spartobacteria*), *Chloroflexi*, *Bacteroidetes* and *Planctomycetes*. *Spartobacteria* in particular are abundant microorganisms in many soil biomes and often the most abundant *Verrucomicrobia* order in soil. Our results also suggest that members of the *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* act in the flow of C through soil trophic levels possibly as predators. Both points illustrate the complexity of soil C dynamics.

2.6 References

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2.7 Figures

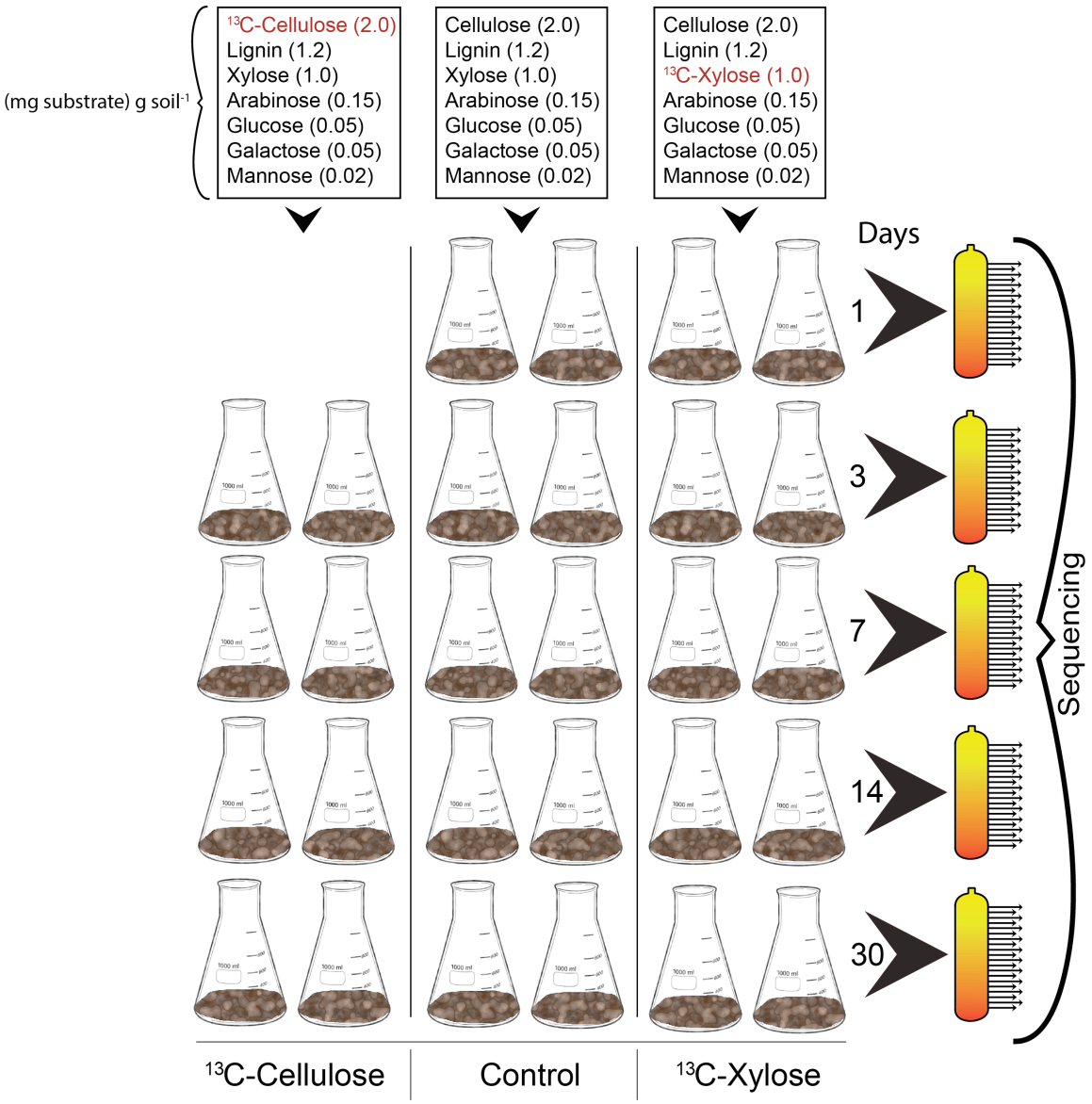

Figure 2.1 The experimental design. A carbon mixture, in addition to inorganic salts and amino acids (not shown here), was added to each soil microcosm where the only difference between treatments is the ^{13}C-labeled isotope (in red). At days 1, 3, 7, 14, and 30 replicate microcosms were destructively harvested for downstream molecular applications. Bulk DNA from each treatment and time point (n = 14) was density separated by centrifugation, then fractionated (orange tubes wherein each arrow represents a fraction from the density gradient). 16S gene amplicons from each fraction were sequenced using next generation sequencing technology.

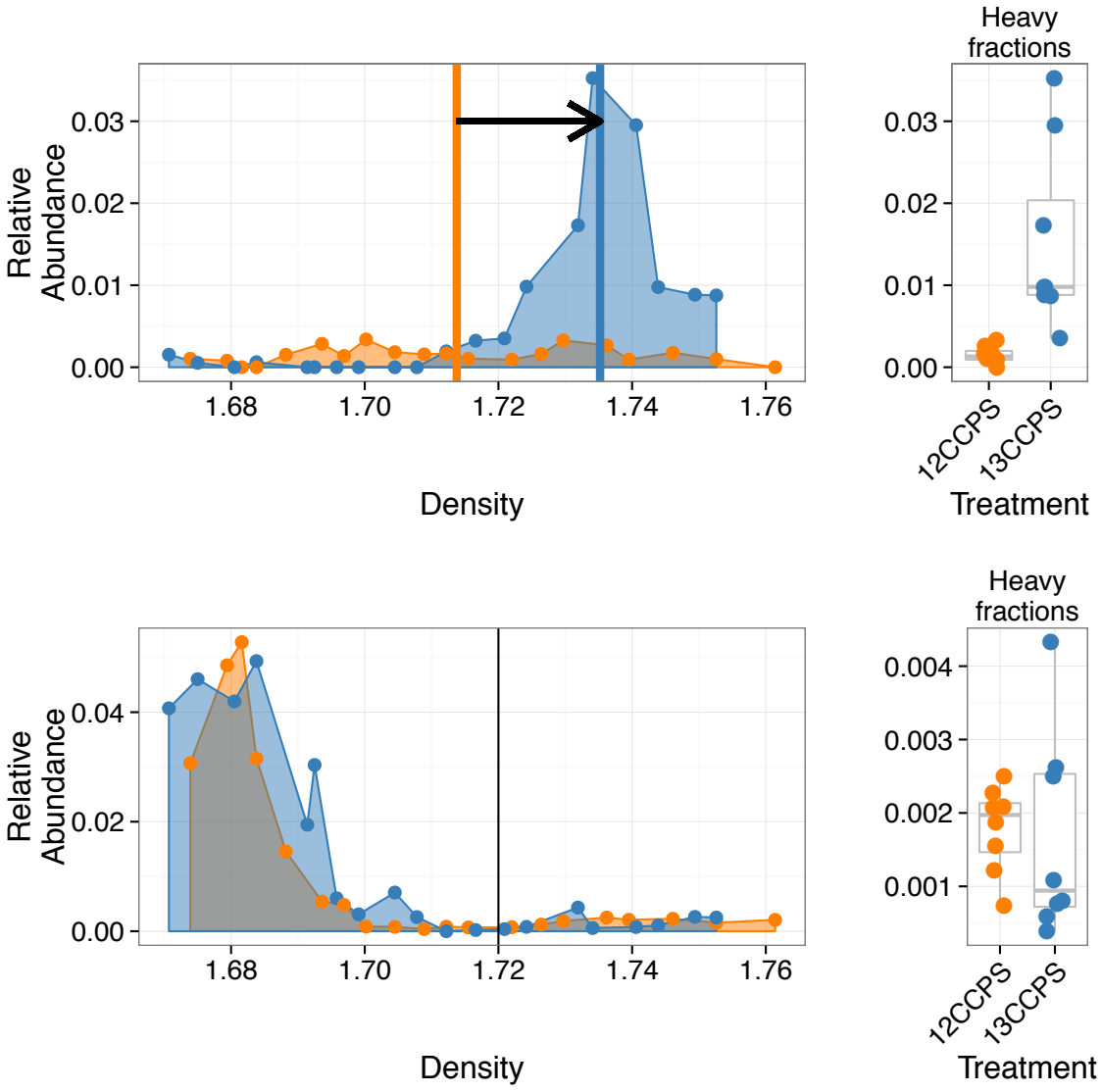

Figure 2.2 Density profile for a single "responder" OTU (top) and a single "non-responder" OTU (bottom) in the ^{13}C-cellulose gradient (blue) and the control gradient (orange). Orange and blue vertical lines show center of mass for each density profile and arrow denotes the magnitude and direction of the BD shift upon labeling. Black vertical line delineates 'light' (<1.72 g mL^{-1}) and 'heavy' (>1.72 g mL^{-1}) fractions. DESeq2 analyses (outlined in methods) were conducted on the 'heavy' fractions. Panel at right shows relative abundance values in the 'heavy' fractions for each gradient. Control (12CCPS) and ^{13}C-cellulose treatment (13CCPS).

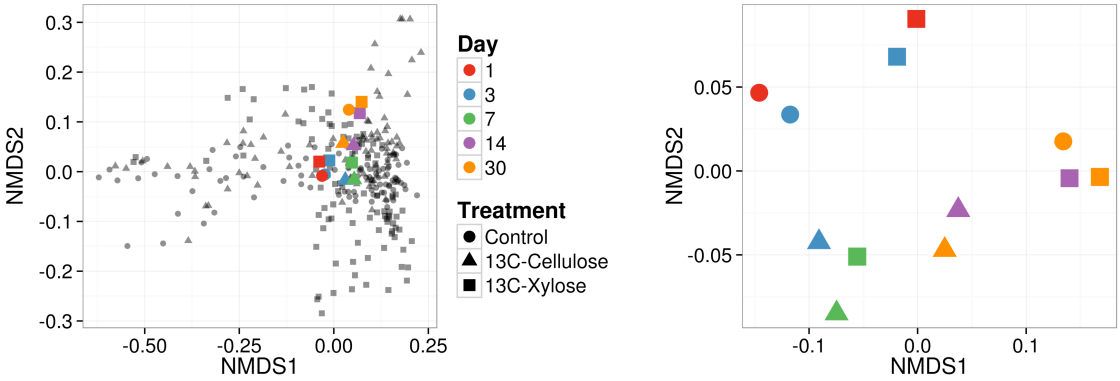

Figure 2.3 NMDS analysis from weighted unifrac distances of 454 sequence data. Left: SIP fractions (grey, described in **Figure 2.7**) and bulk community sequence libraries (colored) of each treatment (control, circle; ^{13}C-cellulose, triangle; ^{13}C-xylose, square) over time. Right: Bulk community sequencing colored by day. Demonstrates community composition changes with time (color), with little variation in composition between different treatments (shapes) within a time point.

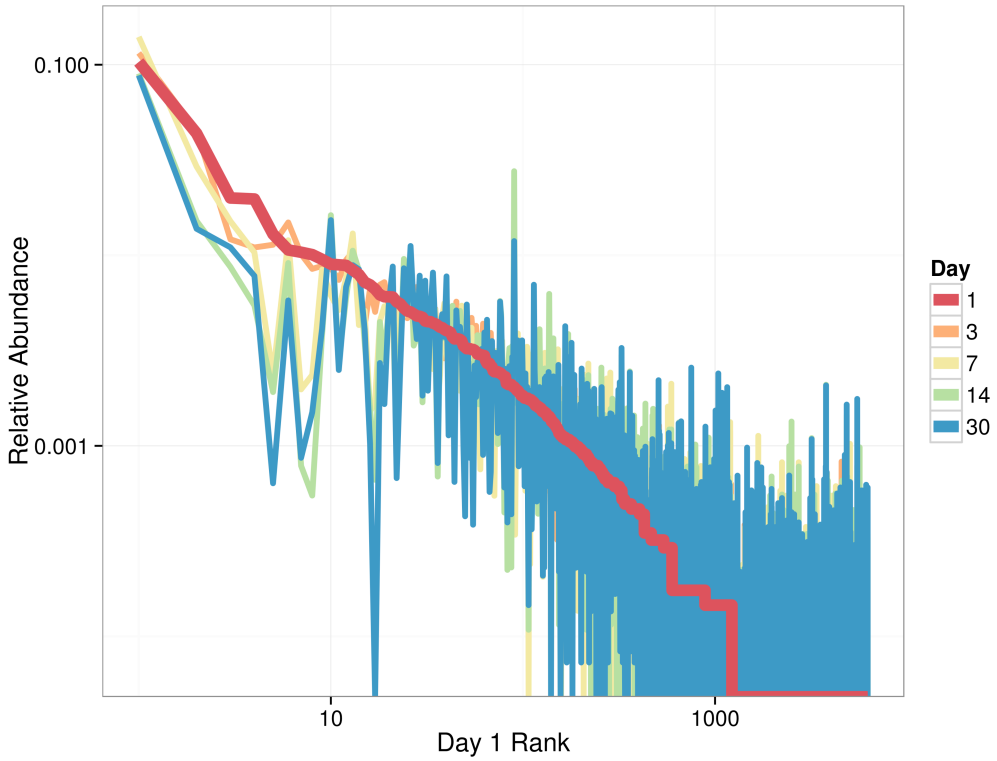

Figure 2.4 Relative abundance of taxa in bulk community over time using each taxa's rank at day 1 (red) as a baseline.

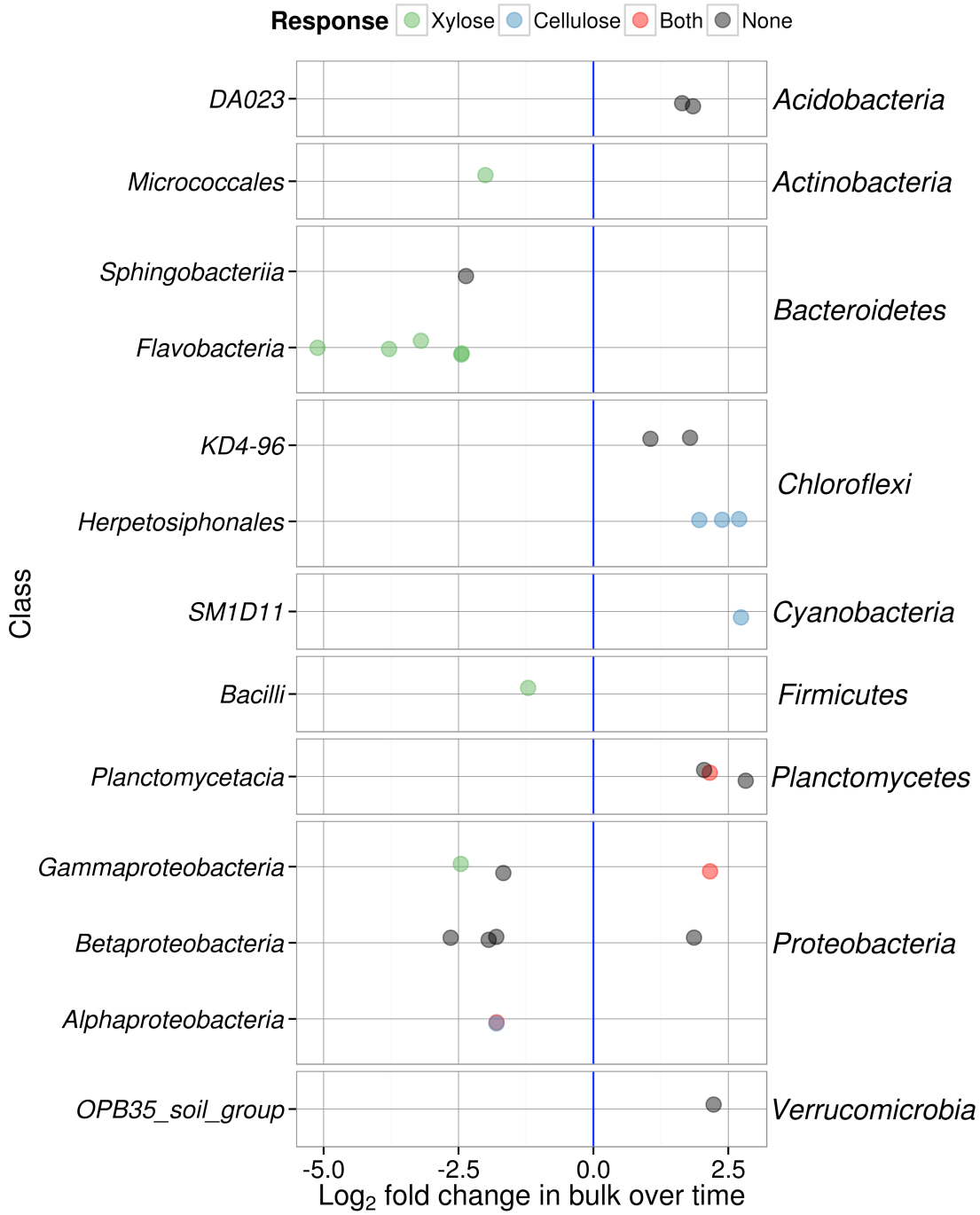

Figure 2.5 Fold change time^{-1} for OTUs that changed significantly in abundance over time in the bulk community. One panel per phylum (phyla indicated on the right). Taxonomic class indicated on the left.

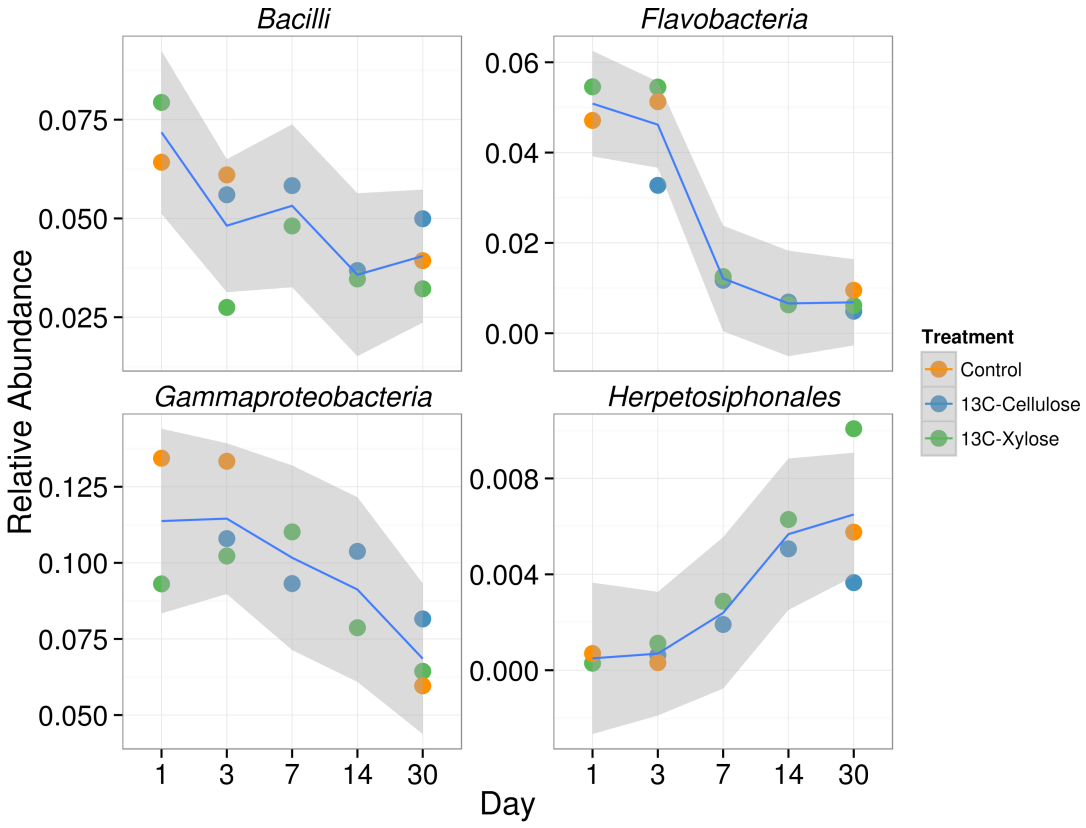

Figure 2.6 Relative abundance versus day for classes that changed significantly in relative abundance with time.

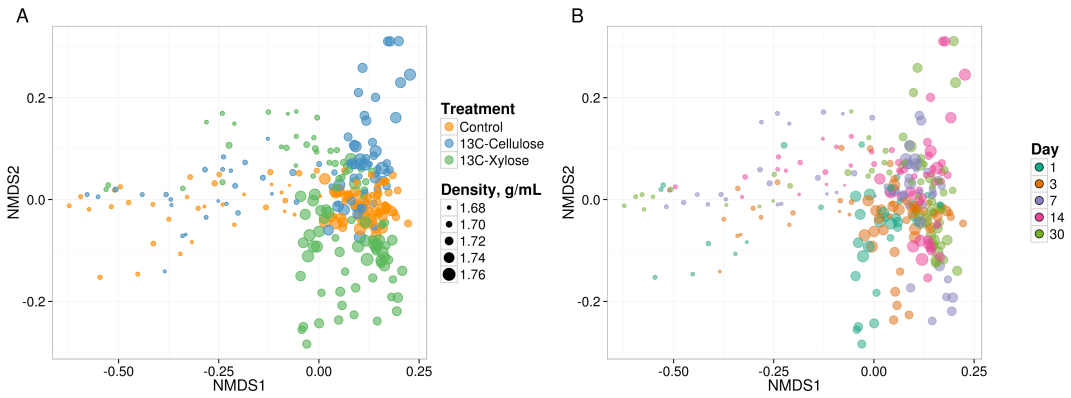

Figure 2.7 NMDS analysis from weighted unifrac distances of 454 sequence data from SIP fractions of each treatment over time. Twenty fractions from a CsCl gradient fractionation for each treatment at each time point were sequenced (**Figure 2.1**). Each point on the NMDS represents the bacterial amplicon composition based on 16S sequencing for a single fraction where the size of the point is representative of the density of that fraction and the colors represent the treatments (A) or days (B).

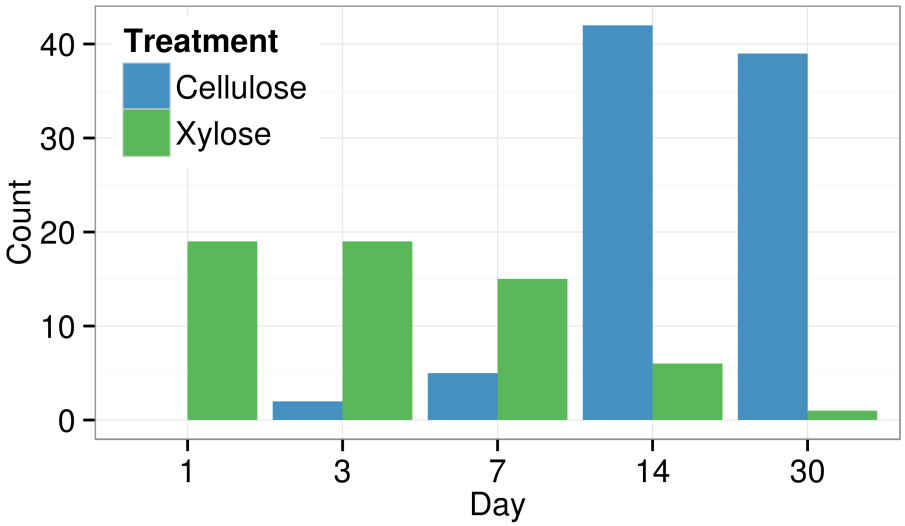

Figure 2.8 Counts of responders for ^{13}C-xylose (green) and ^{13}C-cellulose (blue) over time.

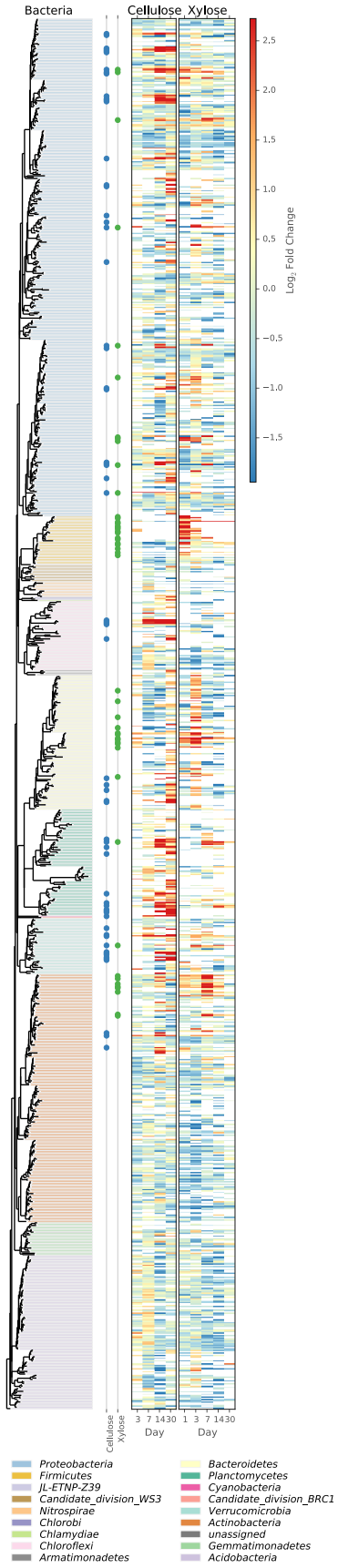

Figure 2.9 16S gene tree. Branches are colored by phylum. ^{13}C-responders for cellulose (blue) and xylose (green) are indicated by a point beside the respective branch. Heatmap demonstrates $\log_2$ fold change of each taxa through the full time series for both treatments (cellulose, left; xylose, right), columns of heatmap are 'day'.

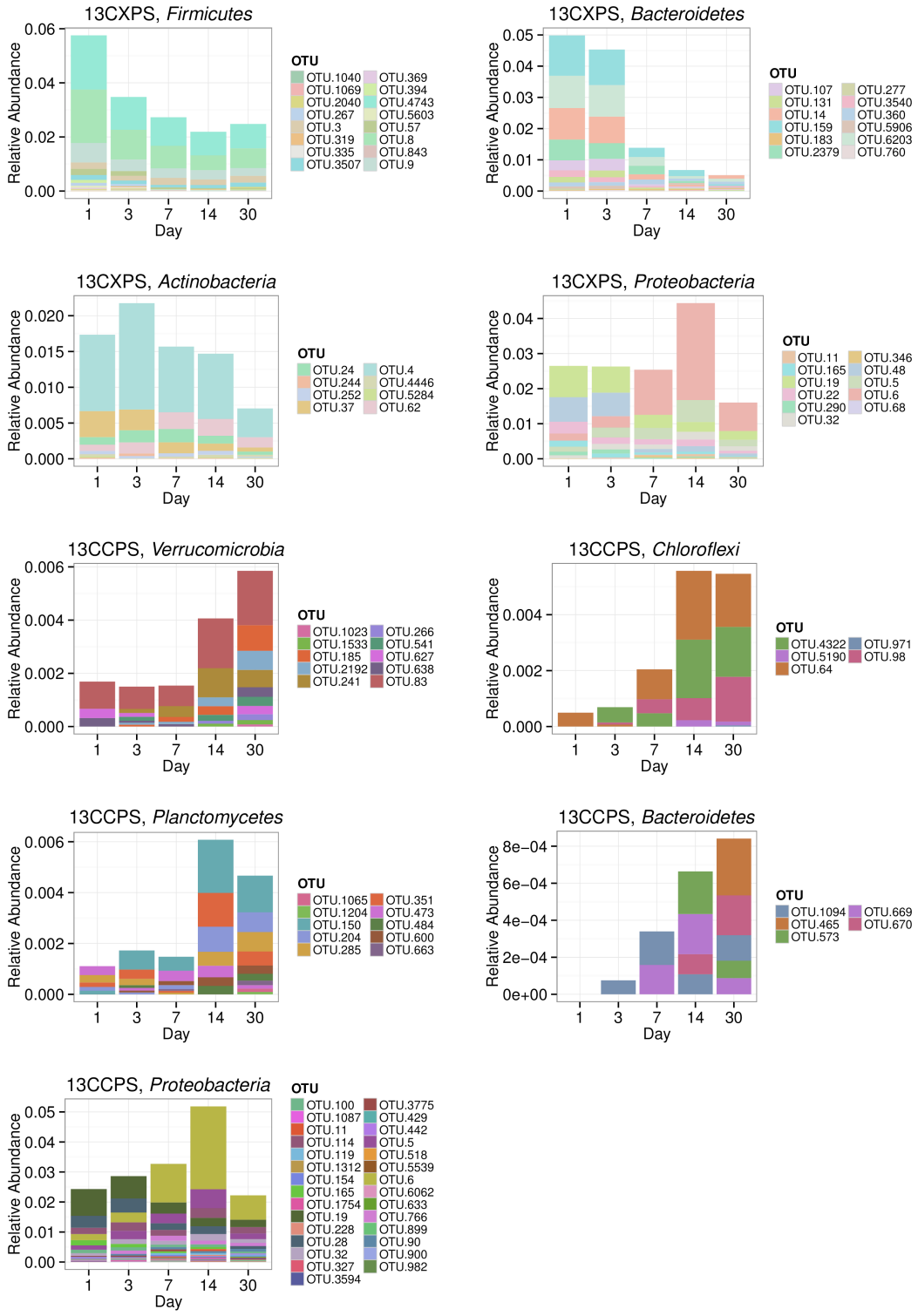

Figure 2.10 Sum of bulk abundances with selected phylum for ^{13}C-xylose (13CXPS, pastel colors) or ^{13}C-cellulose (13CCPS, dark colors) responder OTUs over time.

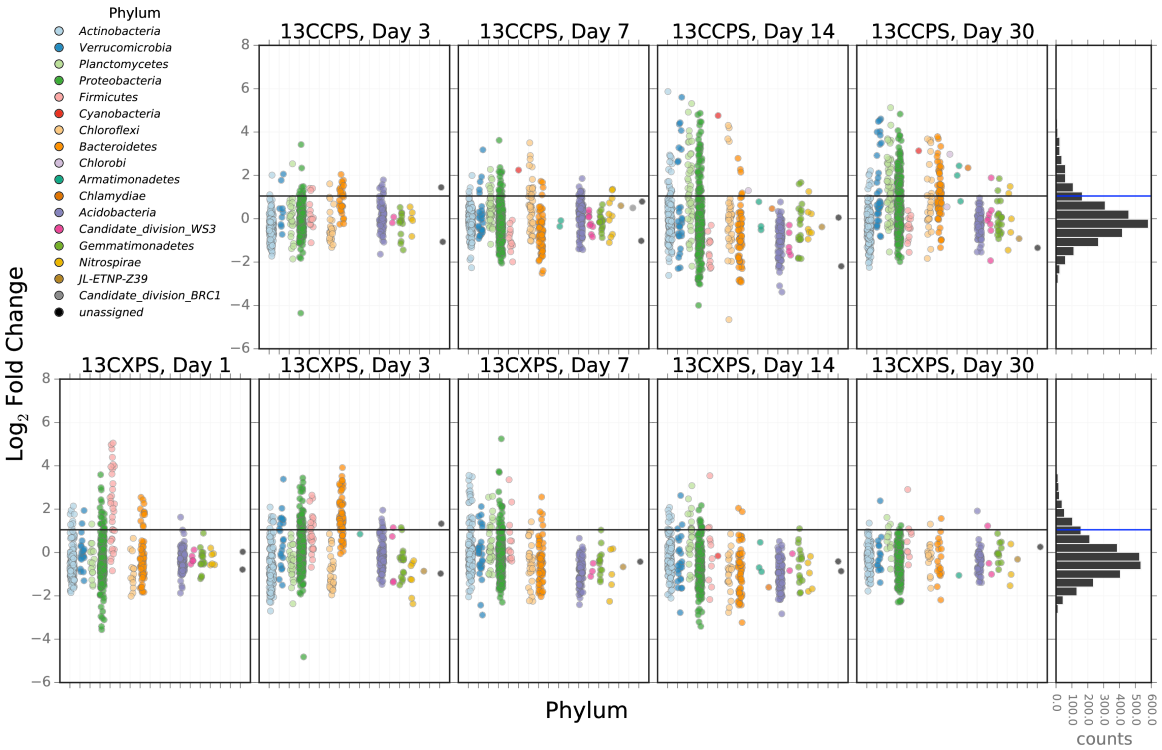

Figure 2.11 Log_2 fold change of ^{13}C-responders in cellulose treatment (top) and xylose treatment (bottom). Log_2 fold change is based on the relative abundance in the experimental treatment compared to the control within the density range 1.7125-1.755 g mL^{-1} (see **Figure 2.2**). Taxa are colored by phylum. 'Counts' is a histogram of log_2 fold change values.

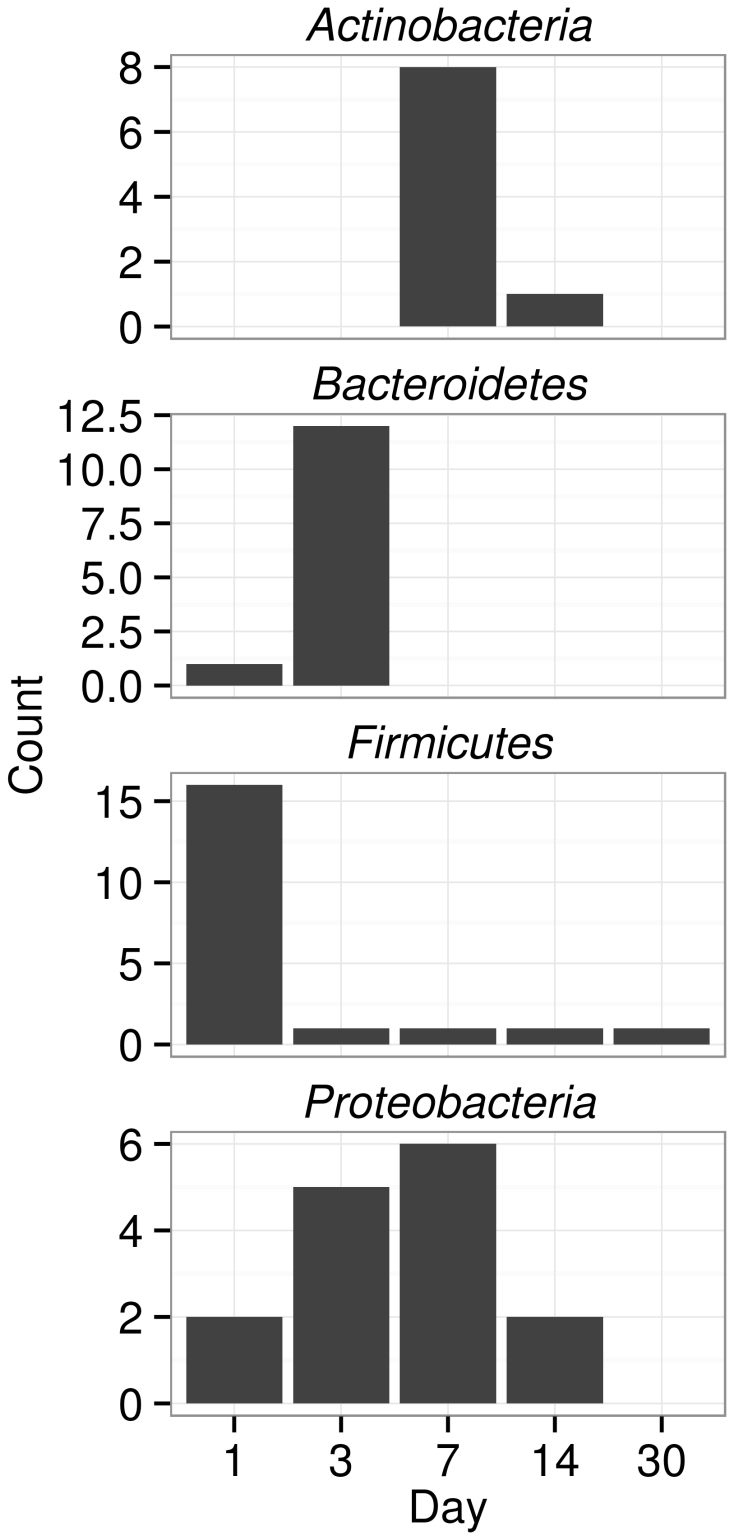

Figure 2.12 Counts of ^{13}C-xylose responders in the *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* at days 1, 3, 7, 14 and 30.

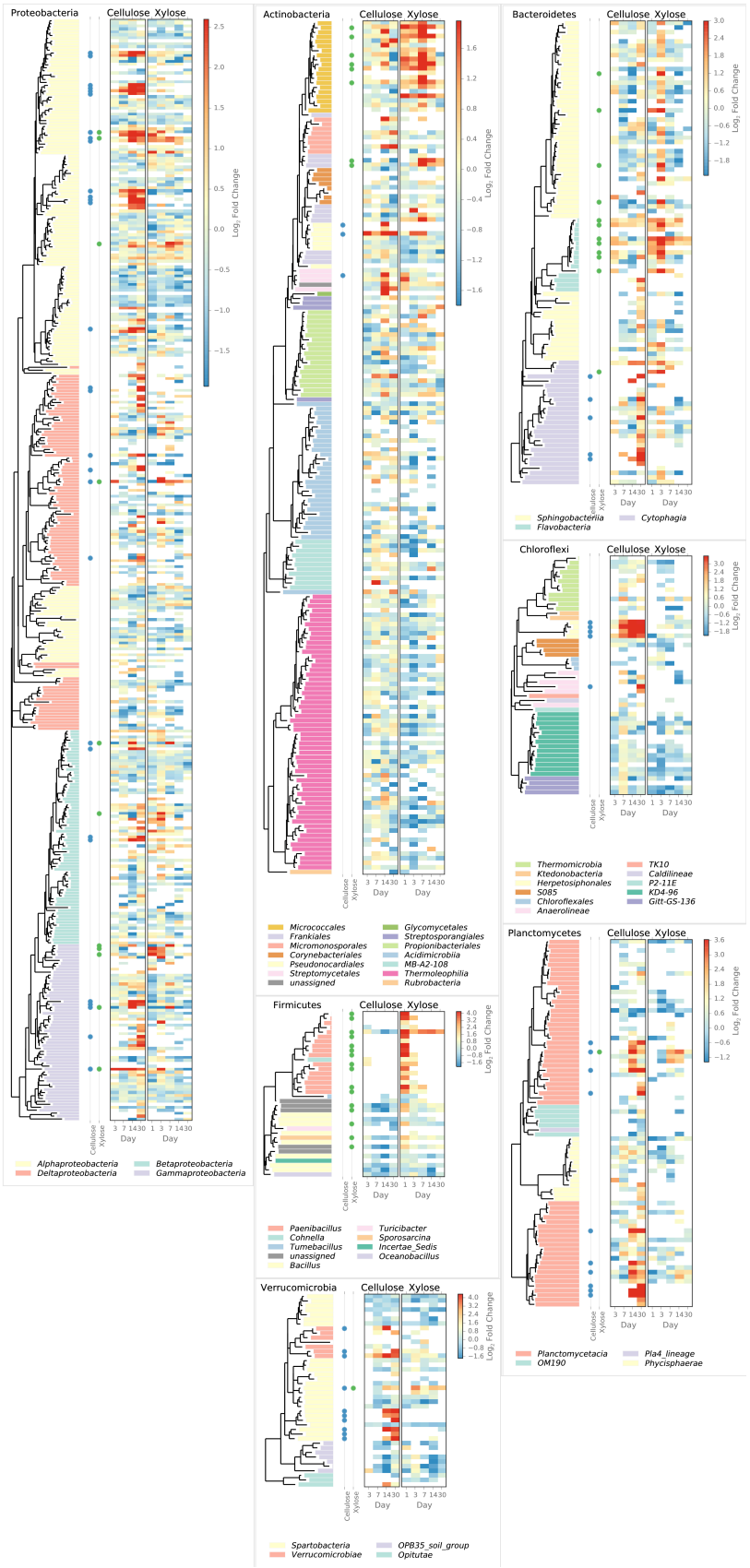

Figure 2.13 Phylum specific 16S gene trees. Heatmap indicates fold change between heavy fractions of labeled gradients versus control gradients. Dots indicate the responders for ^{13}C-xylose (green) or ^{13}C-cellulose (blue).

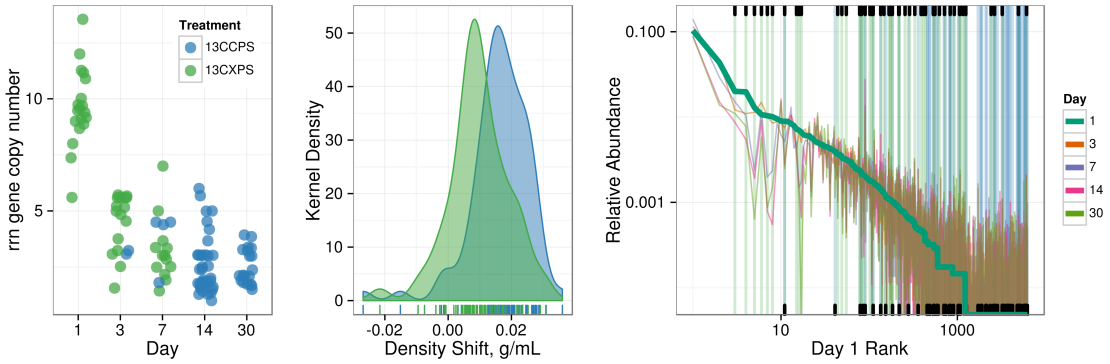

Figure 2.14 ^{13}C-responder characteristics based on estimated *rrn* gene copy number (left), density shift (middle) and rank (right) for ^{13}C-xylose responders (green) and ^{13}C-cellulose responders (blue). Kernel density estimation of ^{13}C-responder's density shift demonstrates degree of labeling for responders for each respective substrate. ^{13}C-responders in rank abundance are labeled by substrate (cellulose, blue; xylose, green). Ticks at top indicate location of ^{13}C-xylose responders in bulk community. Ticks at bottom indication location of ^{13}C-cellulose responders in bulk community. OTU rank was assessed from day 1, bulk samples.

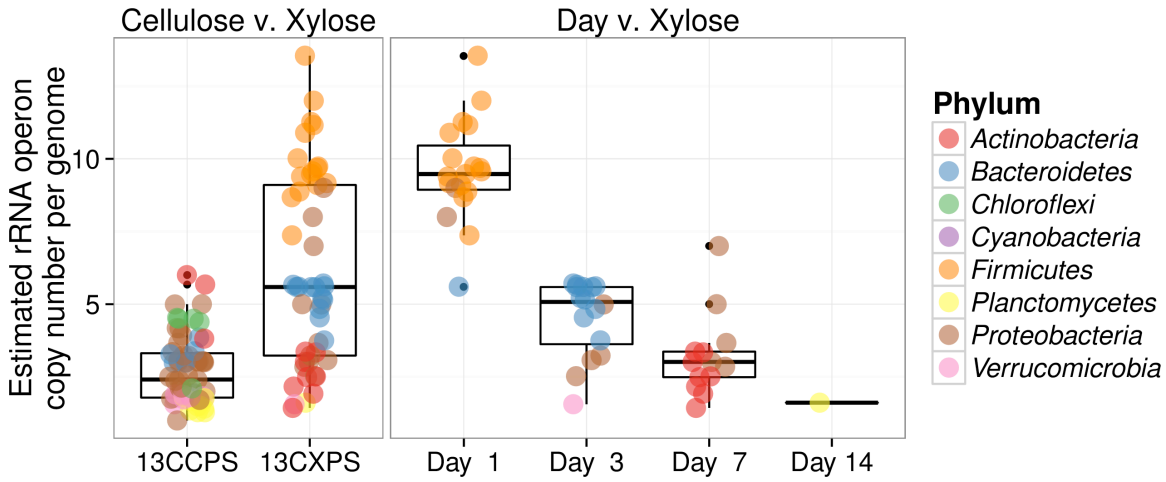

Figure 2.15 Estimated rRNA operon copy number per genome for ^{13}C responding OTUs. Panel titles indicate which labeled substrate(s) are depicted and OTUs are colored by phylum.

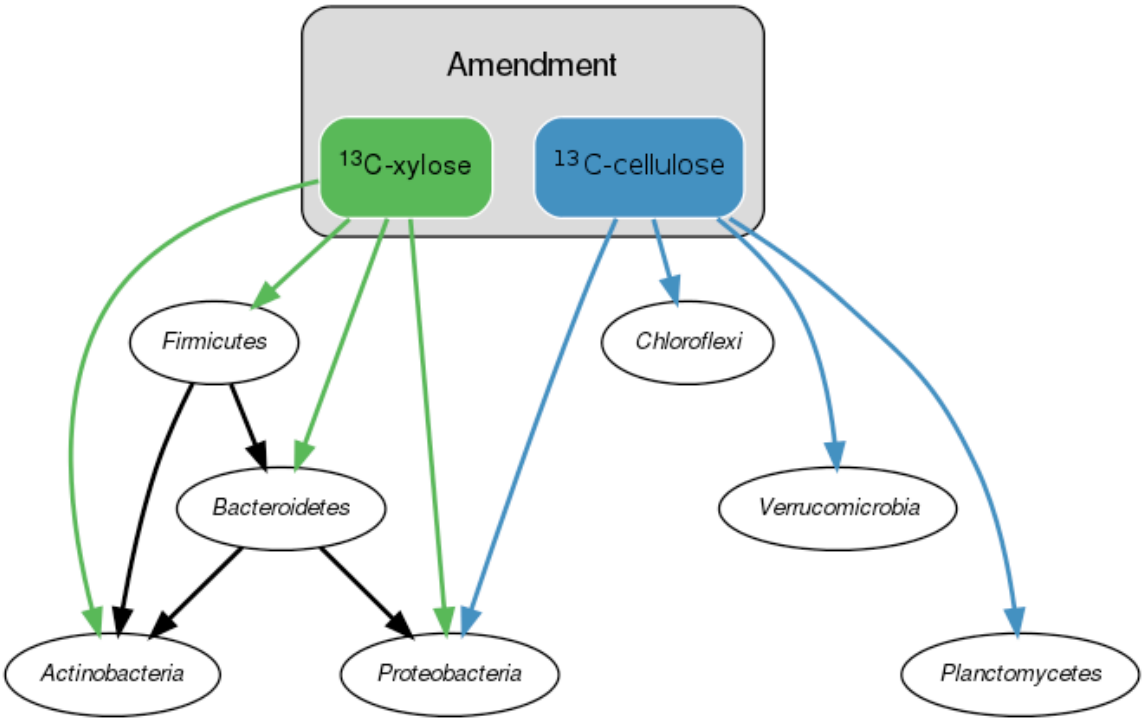

Figure 2.16 Conceptual model of soil food web in this experiment.

2.8 Tables

	Day				
	1	**3**	**7**	**14**	**30**
Xylose	65.3	64	60.7	68.9	71
Cellulose	-	11.3	21.2	37.6	60.2

Table 2.1 Percent of original amount of ^{13}C (0.42 mg xylose-C and 0.88 mg cellulose-C g soil^{-1}) respired from soils over time.

Table 2.2: ^{13}C-xylose responders

OTU ID	**Fold change** [a]	**Day** [b]	**Top BLAST hits** [c]	**BLAST %ID** [c]	**Phylum;Class;Order** [d]
OTU.4446	3.49	7	*Catenuloplanes niger*, *Catenuloplanes castaneus*, *Catenuloplanes atrovinosus*, *Catenuloplanes crispus*, *Catenuloplanes nepalensis*, *Catenuloplanes japonicus*	97.72	*Actinobacteria Frankiales Nakamurellaceae*
OTU.62	2.57	7	*Nakamurella flavida*	100.0	*Actinobacteria Frankiales Nakamurellaceae*
OTU.24	2.81	7	*Cellulomonas aerilata*, *Cellulomonas humilata*, *Cellulomonas terrae*, *Cellulomonas soli*, *Cellulomonas xylanilytica*	100.0	*Actinobacteria Micrococcales Cellulomonadaceae*
OTU.4	2.84	7	*Agromyces ramosus*	100.0	*Actinobacteria Micrococcales Microbacteriaceae*
OTU.37	2.68	7	*Phycicola gilvus*, *Microterricola viridarii*, *Frigoribacterium faeni*, *Frondihabitans sp. RS-15*, *Frondihabitans australicus*	100.0	*Actinobacteria Micrococcales Microbacteriaceae*
OTU.5284	3.56	7	*Isoptericola nanjingensis*, *Isoptericola hypogeus*, *Isoptericola variabilis*	98.63	*Actinobacteria Micrococcales Promicromonosporaceae*
OTU.252	3.34	7	*Promicromonospora thailandica*	100.0	*Actinobacteria Micrococcales Promicromonosporaceae*
OTU.244	3.08	7	*Cellulosimicrobium funkei*, *Cellulosimicrobium terreum*	100.0	*Actinobacteria Micrococcales Promicromonosporaceae*
OTU.760	2.89	3	*Dyadobacter hamtensis*	98.63	*Bacteroidetes Cytophagia Cytophagales*
OTU.14	3.92	3	*Flavobacterium oncorhynchi*, *Flavobacterium glycines*, *Flavobacterium succinicans*	99.09	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.6203	3.32	3	*Flavobacterium granuli*, *Flavobacterium glaciei*	100.0	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.159	3.16	3	*Flavobacterium hibernum*	98.17	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.2379	3.1	3	*Flavobacterium pectinovorum*, *Flavobacterium sp. CS100*	97.72	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.131	3.07	3	*Flavobacterium fluvii*, *Flavobacteria bacterium HMD1033*, *Flavobacterium sp. HMD1001*	100.0	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.3540	2.52	3	*Flavobacterium terrigena*	99.54	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.107	2.25	3	*Flavobacterium sp. 15C3*, *Flavobacterium banpakuense*	99.54	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.277	3.52	3	*Solibius ginsengiterrae*	95.43	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.183	3.31	3	No hits of at least 95% identity	89.5	*Bacteroidetes Sphingobacteriia Sphingobacteriales*

Table 2.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.5906	3.16	3	*Terrimonas sp. M-8*	96.8	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.360	2.98	3	*Flavisolibacter ginsengisoli*	95.0	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.369	5.05	1	*Paenibacillus sp. D75*, *Paenibacillus glycanilyticus*	100.0	*Firmicutes Bacilli Bacillales*
OTU.267	4.97	1	*Paenibacillus pabuli*, *Paenibacillus tundrae*, *Paenibacillus taichungensis*, *Paenibacillus xylanexedens*, *Paenibacillus xylanilyticus*	100.0	*Firmicutes Bacilli Bacillales*
OTU.1040	4.78	1	*Paenibacillus daejeonensis*	100.0	*Firmicutes Bacilli Bacillales*
OTU.57	4.39	1	*Paenibacillus castaneae*	98.62	*Firmicutes Bacilli Bacillales*
OTU.394	4.06	1	*Paenibacillus pocheonensis*	100.0	*Firmicutes Bacilli Bacillales*
OTU.319	3.98	1	*Paenibacillus xinjiangensis*	97.25	*Firmicutes Bacilli Bacillales*
OTU.5603	3.96	1	*Paenibacillus uliginis*	100.0	*Firmicutes Bacilli Bacillales*
OTU.1069	3.85	1	*Paenibacillus terrigena*	100.0	*Firmicutes Bacilli Bacillales*
OTU.843	3.62	1	*Paenibacillus agarexedens*	100.0	*Firmicutes Bacilli Bacillales*
OTU.2040	2.91	1	*Paenibacillus pectinilyticus*	100.0	*Firmicutes Bacilli Bacillales*
OTU.3	2.61	1	*[Brevibacterium] frigoritolerans*, *Bacillus sp. LMG 20238*, *Bacillus coahuilensis m4-4*, *Bacillus simplex*	100.0	*Firmicutes Bacilli Bacillales*
OTU.335	2.53	1	*Paenibacillus thailandensis*	98.17	*Firmicutes Bacilli Bacillales*
OTU.3507	2.36	1	*Bacillus spp.*	98.63	*Firmicutes Bacilli Bacillales*
OTU.8	2.26	1	*Bacillus niacini*	100.0	*Firmicutes Bacilli Bacillales*
OTU.4743	2.24	1	*Lysinibacillus fusiformis*, *Lysinibacillus sphaericus*	99.09	*Firmicutes Bacilli Bacillales*
OTU.9	2.04	1	*Bacillus megaterium*, *Bacillus flexus*	100.0	*Firmicutes Bacilli Bacillales*
OTU.22	2.8	7	*Paracoccus sp. NB88*	99.09	*Proteobacteria Alphaproteobacteria Rhodobacterales*
OTU.5	3.69	7	*Delftia tsuruhatensis*, *Delftia lacustris*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.346	3.44	3	*Pseudoduganella violaceinigra*	99.54	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.32	3.0	3	No hits of at least 95% identity	94.98	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.68	3.74	7	*Shigella flexneri*, *Escherichia fergusonii*, *Escherichia coli*, *Shigella sonnei*	100.0	*Proteobacteria Gammaproteobacteria Enterobacteriales*
OTU.290	3.59	1	*Pantoea spp.*, *Kluyvera spp.*, *Klebsiella spp.*, *Erwinia spp.*, *Enterobacter spp.*, *Buttiauxella spp.*	100.0	*Proteobacteria Gammaproteobacteria Enterobacteriales*

Table 2.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.11	5.25	7	*Stenotrophomonas pavanii*, *Stenotrophomonas maltophilia*, *Pseudomonas geniculata*	99.54	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.48	2.99	1	*Aeromonas spp.*	100.0	*Proteobacteria Gammaproteobacteria aaa34a10*
OTU.241	3.38	3	No hits of at least 95% identity	87.73	*Verrucomicrobia Spartobacteria Chthoniobacterales*

[a] Maximum observed log_2 of fold change.
[b] Day of maximum fold change.
[c] Against Living Tree Project database.
[d] Annotation from Silva database assigned during OTU binning (see methods).

Table 2.3: ^{13}C-cellulose responders

OTU ID	**Fold change** [a]	**Day** [b]	**Top BLAST hits** [c]	**BLAST %ID** [c]	**Phylum;Class;Order** [d]
OTU.862	5.87	14	*Allokutzneria albata*	100.0	*Actinobacteria Pseudonocardiales Pseudonocardiaceae*
OTU.257	2.94	14	*Lentzea waywayandensis*, *Lentzea flaviverrucosa*	100.0	*Actinobacteria Pseudonocardiales Pseudonocardiaceae*
OTU.132	2.81	14	*Streptomyces spp.*	100.0	*Actinobacteria Streptomycetales Streptomycetaceae*
OTU.465	3.79	30	No hits of at least 95% identity	92.73	*Bacteroidetes Cytophagia Cytophagales*
OTU.1094	3.69	30	*Sporocytophaga myxococcoides*	99.55	*Bacteroidetes Cytophagia Cytophagales*
OTU.669	3.34	30	No hits of at least 95% identity	92.69	*Bacteroidetes Cytophagia Cytophagales*
OTU.573	3.03	30	No hits of at least 95% identity	92.76	*Bacteroidetes Cytophagia Cytophagales*
OTU.670	2.87	30	No hits of at least 95% identity	91.78	*Bacteroidetes Cytophagia Cytophagales*
OTU.971	3.68	30	No hits of at least 95% identity	78.57	*Chloroflexi Anaerolineae Anaerolineales*
OTU.64	4.31	14	No hits of at least 95% identity	89.5	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.4322	4.19	14	No hits of at least 95% identity	89.14	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.98	3.68	14	No hits of at least 95% identity	88.18	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.5190	3.6	30	No hits of at least 95% identity	88.13	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.120	4.76	14	No hits of at least 95% identity	94.52	*Cyanobacteria SM1D11 uncultured-bacterium*
OTU.1065	5.31	14	No hits of at least 95% identity	84.55	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.484	4.92	14	No hits of at least 95% identity	89.09	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1204	4.32	30	No hits of at least 95% identity	91.78	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.150	4.06	14	No hits of at least 95% identity	86.76	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.663	3.63	30	No hits of at least 95% identity	90.87	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.473	3.58	14	No hits of at least 95% identity	90.91	*Planctomycetes Planctomycetacia Planctomycetales*

Table 2.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.285	3.55	30	No hits of at least 95% identity	90.87	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.351	3.54	14	No hits of at least 95% identity	91.86	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.600	3.48	30	No hits of at least 95% identity	80.37	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.900	4.87	14	*Brevundimonas vesicularis*, *Brevundimonas nasdae*	100.0	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.1754	4.48	14	*Asticcacaulis biprosthecium*, *Asticcacaulis benevestitus*	96.8	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.119	3.31	14	*Brevundimonas alba*	100.0	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.327	2.99	14	*Asticcacaulis biprosthecium*, *Asticcacaulis benevestitus*	98.63	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.982	4.47	14	*Devosia neptuniae*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1087	4.32	14	*Devosia soli*, *Devosia crocina*, *Devosia riboflavina*	99.09	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.5539	4.01	14	*Devosia subaequoris*	98.17	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.3775	3.88	14	*Devosia glacialis*, *Devosia chinhatensis*, *Devosia geojensis*, *Devosia yakushimensis*	98.63	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.429	3.7	30	*Devosia limi*, *Devosia psychrophila*	97.72	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.766	3.21	14	*Devosia insulae*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.165	3.1	14	*Rhizobium skierniewicense*, *Rhizobium vignae*, *Rhizobium larrymoorei*, *Rhizobium alkalisoli*, *Rhizobium galegae*, *Rhizobium huautlense*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.28	2.59	14	*Rhizobium giardinii*, *Rhizobium tubonense*, *Rhizobium tibeticum*, *Rhizobium mesoamericanum CCGE 501*, *Rhizobium herbae*, *Rhizobium endophyticum*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*

Table 2.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.19	2.44	14	*Rhizobium alamii*, *Rhizobium mesosinicum*, *Rhizobium mongolense*, *Arthrobacter viscosus*, *Rhizobium sullae*, *Rhizobium yanglingense*, *Rhizobium loessense*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.90	2.94	14	*Sphingopyxis panaciterrae*, *Sphingopyxis chilensis*, *Sphingopyxis sp. BZ30*, *Sphingomonas sp.*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.518	4.8	14	*Hydrogenophaga intermedia*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.1312	4.07	30	*Paucimonas lemoignei*	99.54	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.114	2.78	14	*Herbaspirillum sp. SUEMI03*, *Herbaspirillum sp. SUEMI10*, *Oxalicibacterium solurbis*, *Herminiimonas fonticola*, *Oxalicibacterium horti*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.633	3.84	30	No hits of at least 95% identity	89.5	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.3594	3.83	30	No hits of at least 95% identity	90.41	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.442	3.05	30	No hits of at least 95% identity	92.24	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.228	2.54	30	*Sorangium cellulosum*	98.17	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.899	2.28	30	*Enhygromyxa salina*	97.72	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.6	3.62	7	*Cellvibrio fulvus*	100.0	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.6062	4.83	30	*Dokdonella sp. DC-3*, *Luteibacter rhizovicinus*	97.26	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.154	3.24	14	*Pseudoxanthomonas mexicana*, *Pseudoxanthomonas japonensis*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.100	2.66	14	*Pseudoxanthomonas sacheonensis*, *Pseudoxanthomonas dokdonensis*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.1023	4.61	30	No hits of at least 95% identity	80.54	*Verrucomicrobia Spartobacteria Chthoniobacterales*

Table 2.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.266	4.54	30	No hits of at least 95% identity	83.64	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.541	4.49	30	No hits of at least 95% identity	84.23	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.185	4.37	14	No hits of at least 95% identity	85.14	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.2192	3.49	30	No hits of at least 95% identity	83.56	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1533	3.43	30	No hits of at least 95% identity	82.27	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.83	5.61	14	*Luteolibacter sp. CCTCC AB 2010415*	97.72	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.627	4.43	14	*Verrucomicrobiaceae bacterium DC2a-G7*	100.0	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.638	4.0	30	No hits of at least 95% identity	93.61	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*

[a] Maximum observed log_2 of fold change.
[b] Day of maximum fold change.
[c] Against Living Tree Project database.
[d] Annotation from Silva database assigned during OTU binning (see methods).

3 Chapter 3: Variation of amendment composition prompts activity of different cellulose responders within the same soil microbial community

3.1 Abstract

The soil microbial community mediates most of the transformations of freshly added organic matter in soil. These processes are affected by the composition of the organic matter amendment. We used soil microcosms to examine cellulose use by a soil microbial community when cellulose was added with or without additional nutrients. The process of cellulose decomposition was not disrupted by variations in amendment compositions. However, we found the amendments selected for different cellulose responders. The simple (only cellulose) treatment selected for cellulose responders in *Alphaproteobacteria* and *Acidobacteria* that function best at low nutrient conditions. The nutrient conditions of the complex treatment (cellulose + nutrient) better supported cellulolytic microbes belonging to groups with members requiring more nutrients to maintain their unique morphologies and physiologies such as *Planctomycetes* and *Verrucomicrobia*. We believe niche partitioning allows these groups of cellulose responders to co-exist, thus, cellulolytic function remains unchanged under different nutrient conditions.

3.2 Introduction

Microbial ecologists continue to explore whether microbial community structure is important to overall ecosystem functioning. The development of nucleic acid stable isotope probing (SIP) provided a culture-independent means to link

microbial identity to function and has proven to be an invaluable method for elucidating microbial contributions to numerous biogeochemical processes (Dumont, Murrell 2005). In the wild, microbes experience an influx of nutrients as a complex mixture of organic substrates, such as plant biomass. The rate and fate of C can vary depending on how substrates are added (composition, concentration, and timing) to soil (Schneckenberger et al., 2008; Dalenberg, Jager 1989; Sørensen et al., 1996; Bremer, Kuikman 1994).

The soil microbial community dictates plant biomass deconstruction in soil. Within a microbial community different types of plant biomass are degraded dissimilarly (Strickland et al., 2009a; Strickland et al., 2009b). Alternatively, when different soil microbial communities were amended with the same plant biomass, 20% of the variation in decomposition rates could be accounted for by microbial community structure (Strickland et al., 2009a; Strickland et al., 2009b). Microbial importance to ecosystem processes remains debated as we struggle to disentangle the various microbial contributions and strategies when multiple biotic and abiotic factors are involved. As environmental conditions shift there are inherent shifts in the microbial community structure (Allison, Martiny 2008; Kennedy, Smith 1995; Øvreås et al., 1998). Therefore, it is imperative to elucidate microbial contributions to ecosystem function and how ecosystem function may transform as a microbial community structure changes.

Most of our current understanding of microbial contributions to ecosystem processes is from experiments that fall short of natural conditions for how microbes may encounter C in the wild (Ziegler et al., 2005). Conversely, experiments that

successfully reproduce natural conditions are deficient for information regarding which specific carbon (C) substrates are being metabolized (Ostle et al., 2003; Bastian et al., 2009; Fan et al., 2014) and/or the discrete community members mediating the decomposition of those substrates (Jia et al., 2014a; Franzluebbers 1999). We gain a better grasp on structure-function relationships by cataloging the ecological strategies and substrate use of discrete microbes in an intact soil microbial community. For instance, discrete taxa within a microbial community have been shown to be responsible for the utilization of specific C substrates within a mixture of compounds (Singleton et al., 2007; Goldfarb et al., 2011).

Nucleic acid SIP studies are often conducted using a single substrate addition at higher than natural concentrations to ensure label detection (Singleton et al., 2007). The ecosystem scale hypotheses drawn from these data may be less than accurate. Until now, we have been limited by our sensitivity of detection of isotope incorporation and depth of community sampling. Recent technological advances have increased our sensitivity of isotope detection in nucleic acids (e.g. Mayali et al., 2013) and next generation sequencing has increased our phylogenetic resolution. These tools unleash a unique opportunity to assess microbial community structure-function relationships using amendments that better represent natural conditions.

In this study we amended soils with ^{13}C-cellulose either as a sole substrate addition or as part of a complex mixture of organic and inorganic substrates in order to assess whether the composition of the cellulose amendment alters the cellulose-specific response of the microbial community. This experiment employs a high-resolution stable isotope-probing approach (Chapter 2, this dissertation)

where we sequence the 16S rRNA gene amplicons of multiple small fractions from DNA-SIP density gradients. This approach substantially increases the depth of detection of microbial community members throughout the gradient and enables sensitive detection of buoyant density shifts for discrete microbial members resulting from ^{13}C-assimilation. We compare cellulose responders in different amendments and hypothesize that (1) the taxa that assimilate ^{13}C from cellulose will vary depending on the quality of substrate amendments, (2) the taxa assimilating ^{13}C-cellulose in the complex treatment will incorporate more ^{13}C from cellulose than the ^{13}C-assimilating taxa in the simple treatment, and (3) more cellulose will be degraded in the complex treatment that is rich in nutrients than the simple treatment that contains only cellulose.

3.1 Methods

3.1.1 Soil collection and preparation

Soils were collected from an organic farm in Penn Yan, New York. These soils are characterized as Honoeye/Lima (Berthrong et al., 2013). Cores (5 cm diameter x 10 cm depth) were collected in duplicate from six different randomized sampling locations around the field using a slide hammer bulk density sampler (coordinates: (1) N 42° 40.288' W 77° 02.438', (2) N 42° 40.296' W 77° 02.438', (3) N 42° 40.309' W 77° 02.445', (4) N 42° 40.333' W 77° 02.425', (5) N 42° 40.340' W 77° 02.420', (6) N 42° 40.353' W 77° 02.417') on November 21, 2011. Cores were all sieved through a 2 mm sieve, homogenized by mixing, and stored at 4°C until the start of the experiment (within 1-2 weeks of collection). Carbon and nitrogen content were

determined previously for these soils and are 12.15 (± s.d. 0.78) mg C g^{-1} dry soil and 1.16 (± s.d. 0.13) mg N g^{-1} dry soil (Berthrong et al., 2013).

3.1.2 Cellulose production

Bacterial cellulose was produced by *Gluconoacetobacter xylinus* grown in Heo and Son (Heo, Son 2002) liquid minimal media made with 0.1% glucose (one batch with ^{12}C- and another with ^{13}C-glucose). Specifically, cellulose (^{12}C and ^{13}C) was produced in 1 L Erlenmeyer flasks containing 100 mL Heo and Son minimal medium inoculated with three colonies of *G.xylinus* grown on Heo and Son 0.1% glucose agar without inositol (using ^{12}C-glucose) at 30°C. Flasks were incubated statically in the dark at 30°C for 2-3 weeks until a thick cellulose pellicule had formed. Cellulose pellicules were collected and washed with two parts 1% Alconox and autoclaved. Cellulose pellicules were purified by repeated (10x) overnight dialysis in 1 L deionized water. Harvested pellicules were dried overnight (60°C) and then cut into pieces and ground using a ball grinder until desired size range (53 μm – 250 μm) was achieved (checked by dry sieving). The size range was based on the size of particulate organic matter in soil (Cambardella, Elliott 1992).

3.1.3 Soil microcosms

An aliquot of soil was dried at 105°C overnight to determine soil moisture content gravimetrically. Microcosms were 250 mL Erlenmeyer flasks (32 total) each containing 10 g approximate dry soil weight of the sieved soil and capped with a butyl rubber stopper to prevent drying. Stoppers were removed for 10 min every 3 days to exchange the headspace with air. Microcosms were preincubated at 25°C for

2 weeks until the soil respiration rate (determined by GCMS measurement of headspace CO_2) had stabilized. Sieving causes a transient increase in soil respiration rate presumably due to the liberation of fresh labile soil organic matter (Datta et al., 2014). Pre-incubation ensures that this labile organic matter is consumed and/or stabilized prior to the beginning of the experiment. Respiration rate (CO_2) began to plateau around 10 days, with no change in rate after that time (data not shown).

Two treatments were established and each treatment had one series of soil microcosms amended with ^{12}C-cellulose and another series amended with ^{13}C-cellulose. The 'simple treatment' received 2 mg cellulose g^{-1} dry weight (d.w.) soil (0.88 mg cellulose-C g^{-1} d.w. soil) followed by sterile water (0.12 mL g^{-1} d.w. soil). The 'complex treatment' received this same cellulose addition plus lignin (1.2 mg g^{-1} d.w. soil) and an aqueous organic matter simulant (0.12 mL g^{-1} d.w. soil). The organic matter simulant included a mixture of organic and inorganic nutrients of fresh organic matter and is described below. The complex treatment amendment constituted (by mass) 5.3 mg g^{-1} d.w. soil, representative of natural concentrations (Schneckenberger et al., 2008). A total of 32 microcosms were established, 8 replicates for each isotope (^{12}C-cellulose or ^{13}C-cellulose) representing each of the two treatments (simple and complex).

The complex treatment amendment was designed based on switch grass biomass composition (Yan et al., 2010; David, Ragauskas 2010) to include (by mass) 38% cellulose, 23% lignin, 20% xylose, 3% arabinose, 1% galactose, 1% glucose, and 0.5% mannose, with the remaining 13.5% mass composed of amino acids (in-house made replica of Teknova Cat#C0705) and basal salt mixture (Murashige and

Skoog, Sigma M5524). This mixture produced a C:N of 10. The volume of the liquid addition (0.12 mL g^{-1} d.w. soil) was chosen to achieve 50% water holding capacity of the soil. Water holding capacity of 50% was chosen to achieve ~70% water filled pore space in these soils based on soil texture, which is the optimal water content for respiration (Linn, Doran 1984).

Two microcosms per series (four series in total, two series per treatment; see description above) were harvested at days 7, 14 and four microcosms per series were harvested at day 30. Harvested soils were stored at -80°C. Isotopic analysis was performed using soil samples from each treatment series and time point. Isotopic analyses were conducted at the Cornell University Stable Isotope Laboratory.

3.1.4 Nucleic acid extraction

Only nucleic acids from soils harvested at day 30 were extracted as described here. Nucleic acids were extracted from 0.25 g soil using a modified Griffiths procotol (Griffiths et al., 2000). Cell lysis was performed by bead beating for 1 min at 5.5 m s^{-1} in 2 mL tubes containing 0.5 g of 0.1 mm diameter silica/zirconia beads (pretreated at 300°C for 4 hours to remove RNases), 0.5 mL extraction buffer (240 mM phosphate buffer and 0.5% N-lauryl sarcosine), and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1) for 1 min at 5.5 m s^{-1}. After lysis, 85 µL 5 M NaCl and 60 µL 10% hexadecyltriammonium bromide (CTAB)/0.7 M NaCl were added, vortexed, chilled for 1 min on ice, and centrifuged at 16,000 x g for 5 min at 4°C. The aqueous layer was transferred to a new tube and reserved on ice. To increase DNA recovery, the soil pellet was back extracted with 85 µL 5 M NaCl and 0.5 mL extraction buffer.

The aqueous extract was washed with 0.5 mL chloroform:isoamyl alcohol (24:1). Nucleic acids were precipitated by addition of 2 volumes polyethylene glycol solution (30% PEG 8000, 1.6 M NaCl) and a 2 hr incubation on ice, followed by centrifugation at 16,000 x g, 4°C for 30 min. The supernatant was discarded and nucleic acid pellet was washed with 1 mL ice cold 70% EtOH. The nucleic acid pellet was air dried, resuspended in 50 µL TE and stored at -20°C. DNA was size selected (>4 kb) to prepare nucleic acid extracts for isopycnic centrifugation as previously described (Buckley et al., 2007). Briefly, DNA was size separated using 1% low melt agarose gel, bands >4 kb were excised then purified from the gel using β-agarase I enzyme extraction per manufacturers protocol (New England Biolab, M0392S). Final resuspension of DNA pellet was in 50 µL TE.

3.1.5 Isopycnic centrifugation and fractionation

For nucleic acids extracted from each treatment at day 30, isopycnic gradients were setup using a modified protocol (Neufeld et al., 2007). A cesium chloride (CsCl) density gradient solution of an average density 1.69 g mL^{-1} was used to separate ^{13}C-labeled and unlabeled (^{12}C) DNA. The gradient buffer (pH 8.0) used for the density gradient solution was composed of 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl. Each centrifuge tube was loaded with the CsCl density gradient solution and approximately 5 µg of DNA, then centrifuged on a Beckman Coulter Optima™ MAX-E ultracentrifuge using a TLA-110 fixed-angle rotor for 66 h at 55,000 rpm and room temperature (RT).

Fractions of ~100 µL were collected from below the centrifugation tube by displacing the DNA-CsCl-gradient buffer solution in the tube with water using a

syringe pump at a flow rate of 3.3 μL s^{-1} (Manefield et al., 2002) into Acroprep™ 96 filter plate (Pall Life Sciences, PN: 5035,). The refractive index (R_i) of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described (Buckley et al., 2007) to measure a volume of 5 μL. The R_i was corrected to account for the R_i of the gradient buffer using the equation $[R_{i\ corrected}] = [R_{i\ observed}] - ([R_{i\ buffer}] - 1.3333)$. Then the buoyant density was calculated from the $[R_{i\ corrected}]$ using the equation $\rho = a\eta - b$, where ρ is the density of the CsCl (g ml^{-1}), η is the $[R_{i\ corrected}]$, and *a* and *b* are coefficient values of 10.9276 and 13.593, respectively, for CsCl at 20°C (Birnie 1978).

Each well in the Acroprep filter plate contained a single fraction from a CsCl gradient. The collected DNA fractions were purified by washing the Acroprep filter wells five times with 200 μL TE followed by a 10 min centrifugation at 500 x g. Finally, 50 μL TE was added to each well then resuspended DNA was pipetted off the filter into a new microfuge tube.

The number of 16S rRNA genes in each fraction were quantitated by qPCR (Bio-Rad C1000/CFX96 thermocycler) as described previously (Berthrong et al., 2013) using 12.5 μL QuantiFast SYBR green PCR master mix (Qiagen, Valencia, CA; 204056), 1.25 μL 10 μM 515F primer (5'-GTGCCAGCMGCCGCGGTAA -3'), 1.25 μL 10 μM 806R primer (5'- GGACTACHVGGGTWTCTAAT -3'), and 1:100 dilution of DNA template. To estimate the abundances of rRNA gene copies, we used standard curves from 10-fold serial dilutions of 16S rRNA gene amplicons generated from *Klebsiella pneumoniae* using the same primers. The thermocycler conditions

for amplification were 95°C for 5 min followed by 40 cycles of 95°C for 10 s, 53°C for 30 s, and 72°C for 30 s, followed by a final elongation at 72°C for 5 min.

3.1.6 DNA sequencing

For each gradient, ~20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL^{-1}. A total of 4 gradients (80 fractions) and their corresponding bulk DNA extraction (after size selection, described above) were amplified for sequencing. Barcoded 454 primers were designed using 454-specific adapter B, 10 bp barcodes (Hamady et al., 2008), a 2 bp linker (5'-CA-3'), and 806R primer for reverse primer (BA806R, described above); and 454-specific adapter A, a 2 bp linker (5'-TC-3'), and 515F primer for forward primer (BA515F, described above). Each fraction was PCR amplified in triplicate using 0.25 μL 5 U μL^{-1} AmpliTaq Gold (Life Technologies, Grand Island, NY; N8080243), 2.5 μL 10X Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2.5 μL 25 mM $MgCl_2$, 4 μL 5 mM dNTP, 1.25 μL 10 mg mL^{-1} BSA, 0.5 μL 10 μM BA515F, 1 μL 5 μM BA806R, 3 μL H_2O, and 10 μL 1:30 DNA template. The same thermocycler conditions were used as described above except 22 cycles were used instead of 40.

Amplification products were checked by 1% agarose gel electrophoresis. Samples were normalized either using Quant-IT pico green quantification (Life Technologies, Grand Island, NY; P7589) and manual calculation or by SequalPrep™ normalization plates (Invitrogen, Carlsbad, CA; A10510), then pooled in equimolar concentrations. A final purification of pooled DNA was performed via gel extraction from a 1% agarose gel using Wizard SV gel and PCR clean-up system (Promega, Madison, WI; A9281) per manufacturer's protocol. Amplicons were sequenced on

Roche 454 FLX system using titanium chemistry at Selah Genomics (formerly EnGenCore, Columbia, SC).

3.1.7 Post sequencing analysis

Sequence quality control

Sequences were initially screened by maximum expected errors at a specific read length threshold (Edgar 2013), which has been shown to be as effective as denoising with respect to removing pyrosequencing errors. Specifically, reads were first truncated to 250 nucleotides (nt) (all reads shorter than 250 nt were discarded) and any read that exceeded a maximum expected error threshold of 0.5 was removed. After truncation and max expected error trimming, 87% of original reads remained. Forward primer and barcode was then removed from the high quality, truncated reads. Remaining reads were taxonomically annotated using the "UClust" taxonomic annotation framework in the QIIME software package (Edgar 2010; Caporaso et al., 2010) with cluster seeds from Silva SSU rRNA database (Pruesse et al., 2007) 97% sequence identity OTUs as reference (release 111). Reads annotated as "Chloroplast", "Eukaryota", "Archaea", "Unassigned" or "mitochondria" were removed from the dataset. Finally, reads were aligned to the Silva reference alignment provided by the Mothur software package (Schloss et al., 2009) using the Mothur NAST aligner (DeSantis et al., 2006). All reads that did not align to the expected amplicon region of the SSU rRNA gene were discarded. Quality control parameters removed 85,112 reads of 417,925 raw reads (79.6% reads used for analysis).

Sequence clustering

Sequences were distributed into OTUs using the UParse methodology (Edgar 2013). Specifically, OTU centroids (i.e. seeds) were identified using USearch on non-redundant reads sorted by count. The sequence identity threshold for establishing a new OTU centroid was 97%. With USearch/UParse, potential chimeras are identified during OTU centroid selection and are not allowed to become cluster centroids effectively removing chimeras from the read pool. All quality controlled reads were then mapped to cluster centroids at an identity threshold of 97% again using USearch. 97% of quality control reads could be mapped to centroids. Unmapped reads do not count towards sample counts and are removed from downstream analyses. The USearch software version for cluster generation was 7.0.1090.

Phylogenetic analysis

Alignment of OTU centroid SSU rRNA genes was done with SSU-Align which is based on Infernal (Nawrocki et al., 2009; Nawrocki, Eddy 2013). Columns in the alignment that were not included in the SSU-Align covariance models or were aligned with poor confidence (less than 95% of characters in a position had posterior probability alignment scores of at least 95%) were masked for phylogenetic reconstruction. Additionally, the alignment was trimmed to coordinates such that all sequences in the alignment began and ended at the same positions. FastTree (Price et al., 2009) was used to reconstruct the phylogeny.

Community and sequence analysis

OTUs were filtered for ones represented in at least 25% of the samples. The 1,204 OTUs that passed this filtering were used for the following analyses. Nonmetric Multidimensional Scaling (NMDS) uses a distance (or dissimilarity) matrix to find the least stressed relationship between samples in a low dimensional space. Specifically, weighted unifrac (Lozupone, Knight 2005) distances were used for NMDS analyses. The Phyloseq (McMurdie, Holmes 2013) wrapper for Vegan (Dixon 2003) (both R packages) was used to compute sample values along the axes. The ordinations presented here are graphical representations of the sample relationships as determined by NMDS analysis. GGplot2 (Wickham 2009) was used to display sample points in the two-dimensional space. In general, samples in close proximity have more similar microbial composition than samples spaced further away. Adonis tests (Anderson 2001) were done with 1000 permutations to compare community structures.

Identifying OTUs that assimilated ^{13}C into their DNA

DNA-SIP is a culture-independent approach used to reveal identity-function connections (Buckley 2011; Neufeld et al., 2007; Radajewski et al., 2003). Substrate-specific microbial activity is identified on the basis of isotope assimilation into DNA. As the buoyant density (BD) of a macromolecule is dependent on many factors (e.g. GC-content in nucleic acids (Youngblut, Buckley 2014)) in addition to stable isotope incorporation, labeled nucleic acids from one microbial population may have the same BD as unlabeled nucleic acids from another. Therefore, it is imperative to compare results of isotopic labeling to results obtained with unlabeled controls

where everything mimics the experimental conditions except that unlabeled substrates are used. By contrasting heavy gradient fractions from isotopically labeled samples relative to corresponding fractions from controls, the identities of microbes with labeled nucleic acids can be determined.

We used DESeq2, a RNA-Seq differential expression statistical framework (Love et al., 2014), to find OTUs enriched in heavy fractions of labeled gradients relative to corresponding density fractions in control gradients. We use the term "differential abundance", coined by (McMurdie, Holmes 2014), to denote OTUs that have different proportion means across sample classes (in this case the only sample class is labeled:control). CsCl gradient fractions were categorized as 'heavy' or 'light'. The heavy category denotes fractions with density values between 1.7125-1.755 g mL^{-1}. Since we are only interested in enriched OTUs (labeled versus control), we used a one-sided Wald-test for differential abundance (the null hypothesis is the labeled:control proportion mean ratio for an OTU is less than a selected threshold). P-values were corrected with the Benjamini and Hochberg method (Benjamini, Hochberg 1997).

We selected a threshold of 0.75 (or a labeled:control proportion mean ratio of 1.68). Only OTUs present in at least 55% of the density fraction libraries (within the 1.7125-1.755 g mL^{-1} density window) were evaluated with DESeq2. DESeq2 was used to calculate the moderated $\log_2$ fold change of labeled:control proportion mean ratios and corresponding standard errors for the Wald test. Mean ratio moderation allows for reliable ratio ranking such that high variance and likely statistically insignificant mean ratios are appropriately shrunk and subsequently ranked lower

than they would be as raw ratios. Those OTUs that exhibit a statistically significant proportional increase, and pass a false discovery rate of 0.1, in heavy fractions from ^{13}C-labeled samples relative to corresponding controls have increased significantly in buoyant density in response to ^{13}C treatment. OTUs that significantly assimilated ^{13}C into their DNA were identified by BLAST searches that were done with the "blastn" program from BLAST+ toolkit (Camacho et al., 2009) version 2.2.29+. Default parameters were always used and the BioPython (Cock et al., 2009) BLAST+ wrapper was used to invoke the blastn program. Pandas (McKinney 2012) and dplyr (Wickham, Francois 2014) were used to parse and transform BLAST output tables.

Measuring incorporation of ^{13}C assimilating OTUs

Upon labeling, DNA from an organism that incorporates exclusively ^{13}C will increase in BD more than DNA from an organism that does not exclusively utilize isotopically labeled C. We measured the change in the center of mass (deltaCM) from an OTU's density profile between corresponding control and labeled gradients as a metric for ^{13}C assimilation. The center of mass (CM) of DNA increases as its ratio of ^{13}C to ^{12}C increases. An organism that only assimilates C into DNA from a ^{13}C isotopically labeled source, will have a greater ^{13}C:^{12}C ratio in its DNA than an organism utilizing a mixture of isotopically labeled and unlabeled C sources. Therefore the magnitude of the deltaCM is indicative of how much ^{13}C is incorporated into DNA. Wilcoxon rank sum test was used to compare the deltaCM of cellulose-C assimilating OTUs between the two treatments.

3.1.8 Soil isotope data

Soil C and N (mg) were calculated from isotopic analysis (Table 3.1). All treatments started (T_0) with the same amount of cellulose-C (0.89 mg C g^{-1} d.w. soil). Values for cellulose-C remaining in soil at each time point were calculated by subtracting ^{13}Cmg in the ^{12}C-cellulose series (i.e. native soil ^{13}C) from the ^{13}Cmg in the respective ^{13}C-cellulose series; [$^{13}Cmg_{cellulose}$] = [$^{13}Cmg_{13C\text{-}cellulose\ series}$] – [$^{13}Cmg_{12C\text{-}cellulose\ series}$](Figure 3.1-A). These values were used to determine the overall rate of ^{13}C loss from soil (Figure 3.1-B).

3.2 Results

We conducted a nucleic acids SIP experiment to determine whether the composition of substrate amendment alters the identity of the taxa responsible for cellulose degradation. A simple treatment received only cellulose (either ^{12}C or ^{13}C-labeled) while a complex treatment received cellulose (either ^{12}C or ^{13}C-labeled) along with a mixture of inorganic and organic nutrients representative of new organic matter derived from plant biomass. We compared the ^{13}C-assimilating microbial taxa for both treatments via high-throughput DNA sequencing of the SSU rRNA gene content of CsCl gradient fractions. We obtained 417,925 reads from 84 libraries composed of 20 CsCl gradient fractions and 1 bulk soil DNA (unfractionated) for each treatment (n = 4). After quality control there were 332,813 reads that mapped to 6,236 OTUs.

3.2.1 Cellulose use

At day 30, 72% and 60% of the ^{13}C-cellulose was respired from the simple and complex treatments, respectively, as determined using isotopic analysis of the

^{13}C that remained in the soil (Table 3.1, Figure 3.1-A). The overall rate of mineralization of ^{13}C-cellulose to $^{13}CO_2$ from soil was higher in the simple treatment (-0.23mg ^{13}C d^{-1}, R^2: 0.84) than the complex treatment (-0.17mg ^{13}C d^{-1}, R^2:0.97)(Figure 3.1-B). The final C:N ratios of the soils were 9.3 for the simple treatment and 8.9 for the complex treatment (Table 3.1). Overall, total amount of cellulose decomposition between the two treatments is not different but it's also not necessarily the same. More replicates are required to draw any conclusions with confidence.

Interestingly, the total soil C at day 30 increased by 1.33mg C g^{-1} d.w. soil and the total soil N increased by 0.38mg N g^{-1} d.w. soil relative to day 0 values for the simple treatment (Table 3.1). In the complex treatment, total soil C decreased by 0.45mg C g^{-1} d.w. soil and the total soil N increased by 0.20mg N g^{-1} d.w. soil relative to day 0 values (Table 3.1). It is worth noting that these microcosms were incubated as closed systems except for occasional flushing with air to prevent anoxia.

3.2.2 Different cellulose-C assimilating OTUs are detected in the simple versus complex treatment

Neither the difference in amendments nor isotope caused any significant differences in bulk (unfractionated) soil microbial community composition (Adonis test), however, the different amendment compositions prompted the activity of different cellulose-C assimilating OTUs (described as follows). The composition of the amplicon sequences in the heavy gradient fractions from both of the ^{13}C-labeled treatments was significantly different from heavy gradient fractions of both unlabeled controls (Figure 3.2-C; Adonis test; p-value: <0.001, r^2: 0.66). When

comparing only the ^{13}C-simple and ^{13}C-complex treatment, the composition of the amplicon sequences in the heavy fractions were significantly different between the two treatments (Adonis test; p-value: 0.002, r^2: 0.36).

Simple treatment

We contrasted the phylogenetic content of heavy buoyant density fractions from the ^{13}C-simple and equivalent ^{12}C-simple nucleic acid gradients to differentiate ^{13}C-DNA from high G+C content DNA (Pepe-Ranney et al., 2015). The amplicon sequence composition of heavy gradient fractions (>1.725 g mL^{-1}) in the ^{13}C-cellulose treatment varied from corresponding fractions in the ^{12}C-cellulose treatment (control) and this result was significant (Figure 3.2-A, Adonis test; p-value: 0.005, R^2: 0.51).

OTUs that are significantly enriched in the ^{13}C-simple heavy gradient fractions relative to the control gradient are identified as 'responders'. In other words, a 'responder' is an OTU assimilating ^{13}C from cellulose. We identified 29 responders in the simple treatment in the phyla *Proteobacteria* (55%), *Verrucomicrobia* (17%), *Chloroflexi* (10%), *Bacteroidetes* (7%), *Acidobacteria* (3.4%), *Planctomycetes* (3.4%), and *Spirochaetes* (3.4%) (Figure 3.3, Table 3.2). 14 of the 29 responders detected are closely related (>95%) to type stains (Table 3.2). The majority of type strain matches were to *Proteobacteria*. Several OTUs matching the FukuN18 freshwater group belonging to the Verrucomicrobial class *Spartobacteria* had the strongest enrichment in abundance for the simple treatment (Figure 3.4).

There were 17 OTU responders detected exclusively in the simple treatment. Most of the responders unique to the simple treatment were *Alphaproteobacteria* belonging to the *Sphingomonadales* and *Rhizobiales* orders. Other cellulose responders included a Spirochete (*Leptospiraceae* order), an Acidobacterium (*Candidatus Solibacter* order), and an uncultured Verrucomicrobium. *Spirochaetes* have no known ability to degrade cellulose or cellulolytic waste (Berlemont, Martiny 2015), although they have been observed in association with cellulolytic bacteria in anaerobic environments in ruminants (Leschine 1995; Stanton, Canale-Parola 1980). *Acidobacteria*, one of the most abundant soil bacteria, have been implicated to degrade polysaccharides in genomic analyses (Ward et al., 2009) and shown to degrade cellulose in soil (Eichorst, Kuske 2012).

One *Verrucomicrobia* responder identified exclusively in the simple treatment (OTU.400) has no closely related type strains, but shared 100% identity to two verrucomicrobial clones, one from a pristine karstic cave in Germany (Accession FR734396, Rusznyák et al., 2012) and the other from polychlorinated biphenyl-polluted Czech Republic soils (Accession DQ648945, de Cárcer et al., 2007) as determined by a BLASTN (Zhang et al., 2000) search of the NCBI nonredundant database. The sister clades of OTU.400 did not demonstrate assimilation of ^{13}C from cellulose in this study (Figure 3.5).

Complex treatment

As with the simple treatment, the phylogenetic content of heavy buoyant density fractions from the ^{13}C-complex was significantly different from the corresponding heavy fractions of the ^{12}C-complex (Figure 3.2-B, Adonis test; p-

value: <0.001, R^2: 0.58). We identified 18 responders in the complex treatment and they are found in the phylum *Verrucomicrobia* (33%), *Proteobacteria* (22%), *Planctomycetes* (17%), *Chloroflexi* (17%), *Bacteroidetes* (5.5%), and *Cyanobacteria* (5.5%) (Figure 3.3, Table 3.3). Four of the 18 responders are closely related (>95%) to type strains (Table 3.3). The OTUs with the greatest enrichment in abundance for the complex treatment were a novel *Chloroflexi* (previously described, Chapter 2, this dissertation) and two *Verrucomicrobia*; one FukuN18 freshwater group and one from *Verrucomicrobiaceae* (Figure 3.5, Figure 3.4).

The OTU incorrectly annotated as cyanobacteria, falls into the uncultured candidate phylum "Melainobacteria" (Rienzi et al., 2013; Soo et al., 2014) and is discussed in further detail in Chapter 2 of this dissertation.

Cellulose-C assimilating OTUs shared across both treatments

Of the responders identified for the simple treatment (n=29) and the complex treatment (n=18), eight were identified as a responder in both treatments (Figure 3.4). The shared responders belonged to the *Verrucomicrobia* (*Spartobacteria*), *Planctomycetes* (*Planctomycetacie*), *Chloroflexi* (*Herpetosiphonales*), and *Alphaproteobacteria* (*Rhizobiales*)(Figure 3.4). These groups are found in many diverse environments and are known to function under varying environmental conditions (Dua et al., 2013; Jia et al., 2014b; Vanparys et al., 2005; Yoon et al., 2007; Bergmann et al., 2011; Hug et al., 2013; Fuerst, Sagulenko 2011; Neef et al., 1998). Notably, the *Rhizobiales* (*Devosia* gen.) have not been directly observed as using cellulose, but have been detected in environmental studies assessing cellulolytic activity (Verastegui et al., 2014; Talia et al., 2012).

3.2.3 Characteristics of cellulose responders are the same in both treatments

There is no discernable difference in the enrichment of OTU abundance between the two treatments. Measured OTU abundances in the simple and complex treatments reveals a positive linear relationship (Figure 3.6-A). In other words each OTU has a comparable abundance in both treatments (Figure 3.6-A), with few exceptions (Figure 3.6-B).

There is no significant difference in the amount of cellulose-C incorporated by cellulose responders between the two treatments as assessed by deltaCM (see methods for greater detail). DeltaCM is greater for organisms assimilating more ^{13}C; the more ^{13}C an organism assimilates, the greater the preference that organism has for that compound. We tested whether the deltaCM of the responders was significantly greater or less between the two treatments and found no significant difference using the Wilcoxon rank sum test ((Wilcoxon 1945), Figure 3.7-A). We also found no phylum level differences in the deltaCM of responders between the two treatments (Figure 3.7-B).

3.3 Discussion

In soils, the composition of plant biomass is a driver of microbial community structure and function (O'Donnell et al., 2001; Ng et al., 2014). Until recently we were unable to detect ^{13}C assimilation into microbial biomass unless it was strongly labeled, causing SIP studies to be conducted by means of adding an ecologically unrealistic amount of isotopically-labeled substrate in order to achieve a detectable signal. Additionally, the path of specific C substrates through the microbial food web cannot be determined when added as labeled plant biomass, where all chemical

components of the biomass are labeled. Whether added as a single compound or as plant biomass, the high concentrations of amendments fall short of how microbes encounter organic matter in the wild.

Here, we probed a soil microbial community's response to ^{13}C-cellulose amendments in the presence or absence of a complex mixture of organic and inorganic substrates added at ecologically realistic concentrations to determine the consequences of amendment composition on microbial community response. There was no difference in the overall structure of the bulk soil microbial community between the two treatments. However, we found that composition of amendment prompted activity of different ^{13}C-cellulose assimilating microbial community members ('responders') within the same soil microbial community. It was unclear if there was a difference in overall cellulose deconstruction between the two treatments after 30 days despite phylogenetic differences of the cellulose responders detected between the two treatments.

3.3.1 Composition of amendments prompts activity of different cellulose responders

We measured cellulose responders in two nutrient conditions, only cellulose (simple) or cellulose in the presence of additional nutrients (complex). The composition of the amendments added to soil prompted the activity of phylogenetically different cellulose responders as determined by assimilation of cellulose-C. We propose the discrepancies in cellulose responders between the two treatments result from niche partitioning. In this instance, organisms competing for cellulose occupy slightly different ecological niches and/or their means of cellulose acquisition. In this study cellulose responders detected in one treatment and not the

other are active at different nutrient conditions; they are tuned to the specific resource concentrations in the treatment or have some other competitive advantage under those conditions.

The complex treatment conditions may provide various nutrients necessary to maintain a cellulolytic lifestyle (Treseder et al., 2011; Berlemont et al., 2014). Although, the copious nutrients supplied would also enrich for fast-growing bacteria in the microbial community that would be able to outcompete slow-growing bacteria, such as cellulose degraders. Additionally, there is evidence that the presence of labile C may repress cellulolytic enzyme synthesis (Blagodatskaya et al., 2014; Berg, Laskowski 2005). Cellulose responders detected exclusively in the complex treatment, with the exception of the misidentified cyanobacteria (currently no physiological data), share interesting commonalities including an unusual prosthecate stalk morphology and/or membrane-bound intracellular structures (only *Verrucomicrobia* and *Planctomycetes*) (Lee et al., 2009). We are not certain how, if at all, these morphologies may be linked to their response in the complex treatment. These characteristics may confer some advantage under osmotic stress or the biomass elemental stoichiometry of their unique morphology may require more nutrients (Ng et al., 2014).

The nutrient deplete conditions of the simple treatment allow a competitive advantage for cellulolytic bacteria which are generally characterized as slow growing. Cellulolytic bacteria are usually characterized by their ability to thrive when nutrients are scarce (Fontaine et al., 2003). The *Alphaproteobacteria* and *Acidobacteria* responders detected exclusively in the simple treatment may flourish

under the conditions in this treatment due to the lack of nitrogen addition (Cederlund et al., 2014). Relative abundance of *Acidobacteria* has been shown to be negatively correlated to total nitrogen (Cederlund et al., 2014; Fierer et al., 2012; Leff et al., 2012). *Alphaproteobacteria*, on the other hand, were noted to increase with increasing litter additions to soil (Leff et al., 2012) suggesting a positive response to increasing C. We observed the opposite trend, with an increase in *Alphaproteobacteria* in the lower C conditions, in accordance with Cederlund *et al.* (2014).

We identified more ^{13}C-cellulose responders in the simple treatment than the complex treatment (n = 29 and 18, respectively). A decrease in bacterial incorporation of cellulose and plant cell walls was observed when C substrates were added with N (Koranda et al., 2014) and our observations support these previous findings.

3.3.2 Sources of nitrogen in the simple treatment

In the simple treatment, N-limitation may be alleviated via N fixation or biomass turnover (resulting in N release). The water addition accompanying the cellulose addition in the simple treatment could mobilize easily digestible C and N native to the soil. Availability of labile C substrates encourage production of microbial residues such as exo-enzymes, mucous substances, secondary metabolites, and dead tissue which immobilize N; these residues can be recycled by other microorganisms during periods of energy limitation (Engelking et al., 2007). If easily digestible C substrates were mobilized by the addition of water, then this chain reaction could have been set in motion; serving as another potential source of

N for cellulolytic microorganisms. While N sourced from biomass turnover is most certainly occurring, the observed increase in soil N content at the end of the incubation (Table 3.1) would suggest N fixation is also occurring since it is a closed system. The increase in soil C content in the simple treatment suggests that C fixation is also occurring.

3.3.3 Cellulose decomposition as a functionally equivalent process

We find that phylogenetically distinct cellulose responders have no significant difference in the amount of ^{13}C incorporated from cellulose. The deltaCM of all responders, resulting from the incorporation of ^{13}C-cellulose into DNA, were not significantly different between the treatments. This indicates that the preference for cellulose-C by responders is similar regardless of responder identity and composition of amendments. This would suggest that cellulose responders have a similar magnitude of substrate preference for cellulose regardless of the presence of other C substrates (as with the complex treatment). If cellulose responders were using other C substrates (unlabeled) in addition to cellulose (labeled), there would have been a measurably smaller deltaCM in responders from the complex treatment. If a difference in cellulose-C incorporation existed, it would manifest as a difference in ^{13}C assimilation into DNA as has been detected with this method when comparing xylose-C and cellulose-C responders (Chapter 2 in this dissertation). The absence of a significant difference in cellulose-C assimilation suggests high substrate preference by cellulose utilizers. This is not surprising considering the specialized process of cellulose decomposition (Lynd et al., 2002).

We predicted to observe a significant difference in the amount of cellulose degraded between the two different amendment compositions. Other studies have detected a significant difference in microbial community function resulting from variations in amendment composition (e.g. Ng et al., 2014). Specifically, we expected cellulose use in the complex treatment to be higher due to an increase in potential opportunist (i.e. cheater) biomass resulting from the availability of labile C and N in the mixture, but this was not observed. Here, opportunists are considered organisms that do not pay the energetic cost of producing cellulolytic enzymes but receive the benefits of cellulases by using cellulose breakdown products. In some cases, opportunists may work with cellulose degraders in synergistic interactions where the opportunist aids in a timely removal of hydrolysis products (Perez 2002). High concentrations of cellulolytic waste inhibit cellulase synthesis and activity (Berlemont2015); shutting down cellulose deconstruction. In a natural setting these synergistic relationships may be more intimately connected especially during plant cell wall degradation, which relies on the activity of many microbes to disentangle the mesh of C substrates. More replicates over longer time scales would help discern differences, if any, in cellulose decomposition resulting from variation in amendment composition.

Our data suggests cellulose decomposition is a functionally equivalent process, that is, phylogenetic differences do not confer functional differences. Specifically, the inability to distinguish any defining differences in the total amount of cellulose used or substrate preference between the two cellulose responder

groups, despite phylogenetic differences, suggests a degree of cellulose-specific functional equivalency.

3.3.4 Consequences of microbial biodiversity

A multitude of cellulose degrading microorganisms are able to co-exist through niche partitioning (discussed above). The process of cellulose decomposition was not disrupted despite a change in environmental conditions (nutrient addition). The limited similarity in cellulose responder phylogeny between the two treatments suggests that niche partitioning maintains cellulose-specific function over a range of nutrient conditions.

The residence time of cellulose-C may be more sensitive to the identity of its microbial metabolizer. Discrete OTUs will differentially metabolize cellulose resulting in variation in its final form (ie. fate) depending on the utilizer (Kindler et al., 2006). The fate of this C will ultimately dictate its soil residence time, which could have global implications. The variation in taxonomic groups processing cellulose in these two different amendments likely indicates a different fate of the cellulose-C. This difference in C fate would result in variation of soil residence time, suggesting that we are not accurately estimating C residence time using SIP studies with single substrates. In the future, we may gain a better understanding of the fate of C in soil by a metabolomics approach employing isotopes.

3.4 Conclusion

In this study we demonstrate that variation in amendment composition prompts the activity of different cellulose degrading members of the soil microbial

community without a loss or change in cellulose-specific function. Niche partitioning of cellulose responders ensures cellulose-cycling in soil is maintained under varying nutrient conditions. Taxa tuned to low nutrient conditions were detected in the simple treatment, while a different group of taxa able to function at higher nutrient conditions were detected in the complex treatment. Cellulose responders detected in both treatments are known to function under a range of environmental conditions resulting in resource overlap with low and high nutrient cellulose responders. Niche partitioning enables the co-existence of organisms competing for the same resource and facilitates maintaining biodiversity. The data presented here highlights the importance of microbial biodiversity for cellulose cycling in soil. Microbial biodiversity acts to buffer microbial community functionality by maintaining activity in a wide range of environmental conditions (e.g. N limitation).

In the future, we should continue to work towards measuring substrate-specific activities of discrete OTUs under varying environmental conditions and time scales. For instance, in a previous study using the same soils and amendments we detected *Cellvibrio* as one of the strongest cellulose responders between days 3-14 (Chapter 2, this dissertation). Had we measured cellulose responders only at day 30 (like we did in this study), we could have missed a potentially important contributor to cellulose-C cycling. Furthermore, it is becoming increasingly apparent that in order to better assess the ecosystem-scale importance of microbial community membership and activities, we not only need to identify the discrete microorganisms mediating C-cycling but also need to determine the fate of C given different microbial mediators using metabolomics.

3.5 References

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3.6 Figures

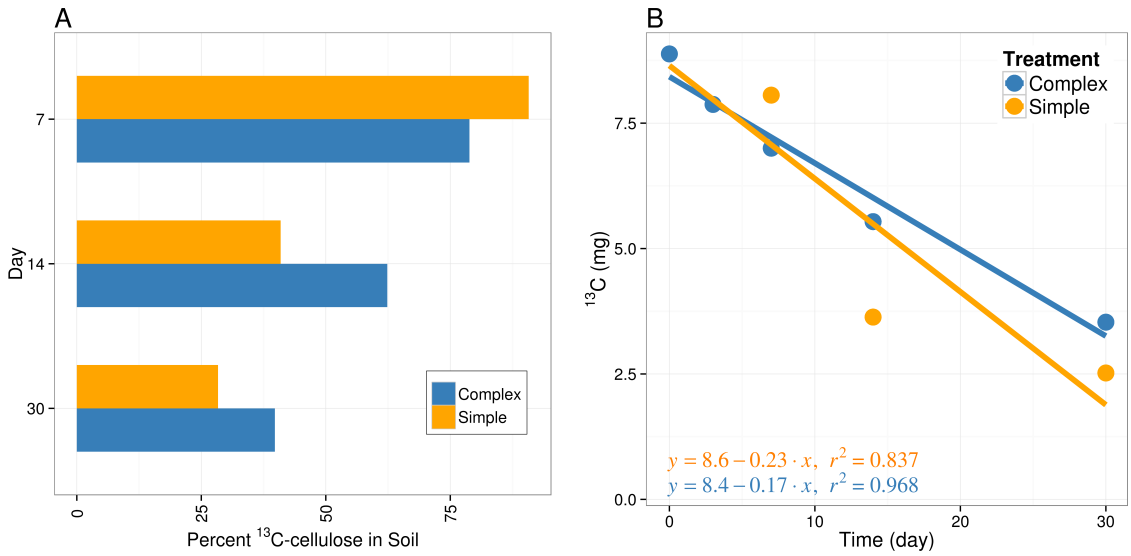

Figure 3.1 Percent of ^{13}C from cellulose in soil the complex treatment (blue) and simple treatment (orange) over time (A). Rate of ^{13}C-cellulose loss from soil in the simple and complex treatments (B).

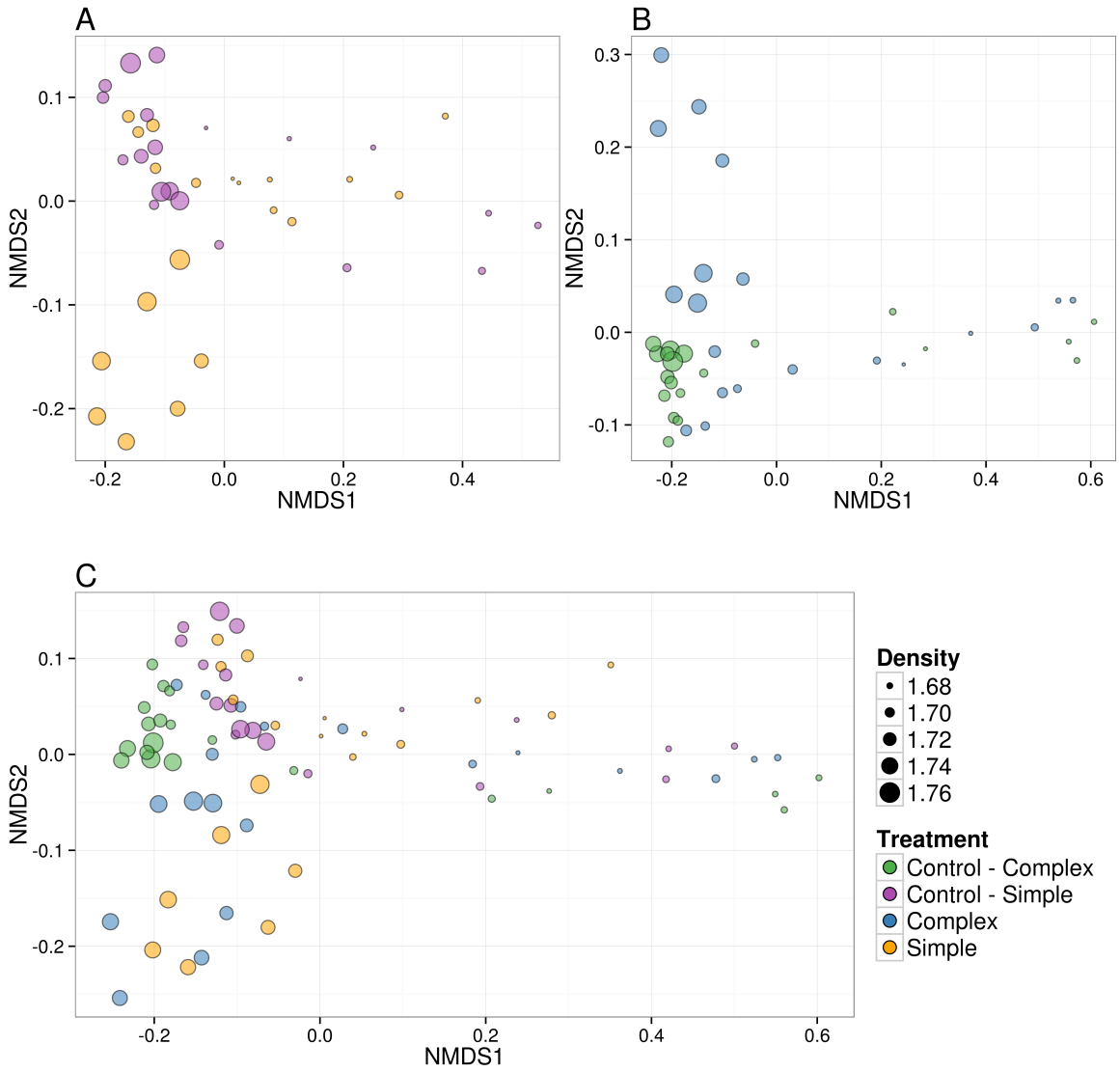

Figure 3.2 NMDS analysis of 16S rRNA gene amplicon composition from density gradient fractions from: (A) the simple treatment (control, purple; ^{13}C-cellulose, orange), (B) the complex treatment (control, green; ^{13}C-cellulose, blue), and (C) both treatments. Fractions are sized according to its buoyant density. When members of the microbial community assimilate ^{13}C into their DNA their buoyant density increases. As a result, heavy density fractions (points with a 1.72 density or greater) from microcosms amended with ^{13}C-cellulose diverge from respective unlabeled-cellulose heavy density fractions.

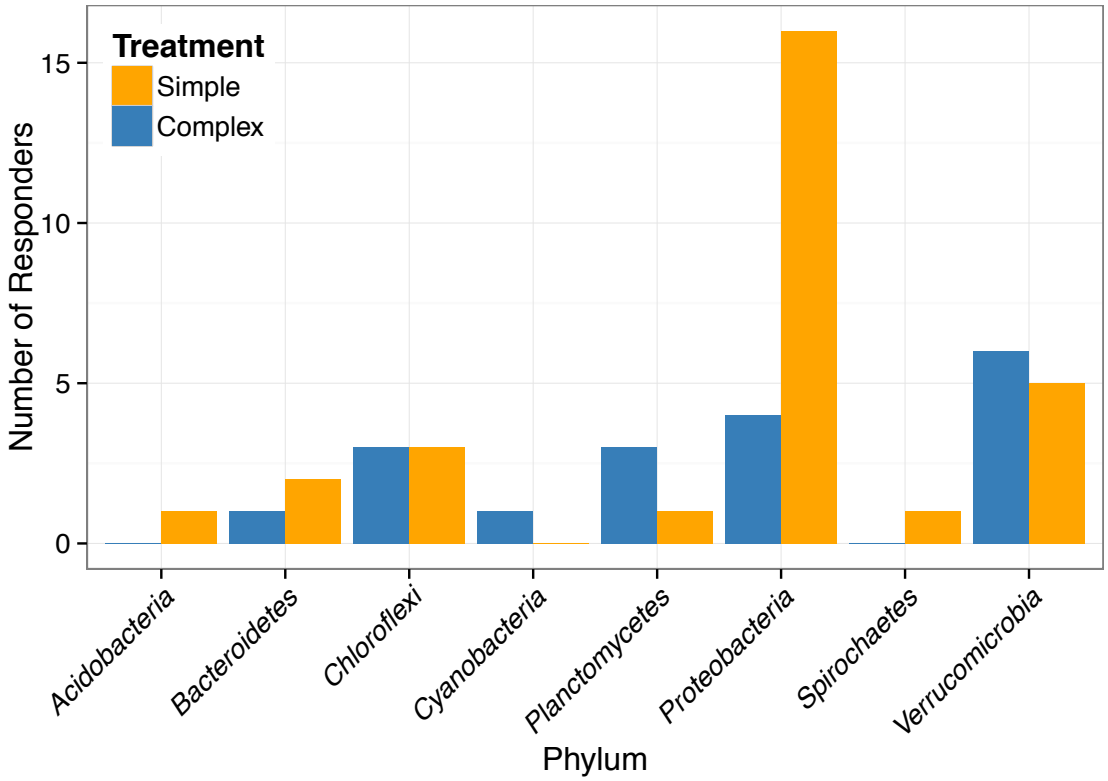

Figure 3.3 Counts of responders from complex treatment (blue) and the simple treatment (orange) by phylum.

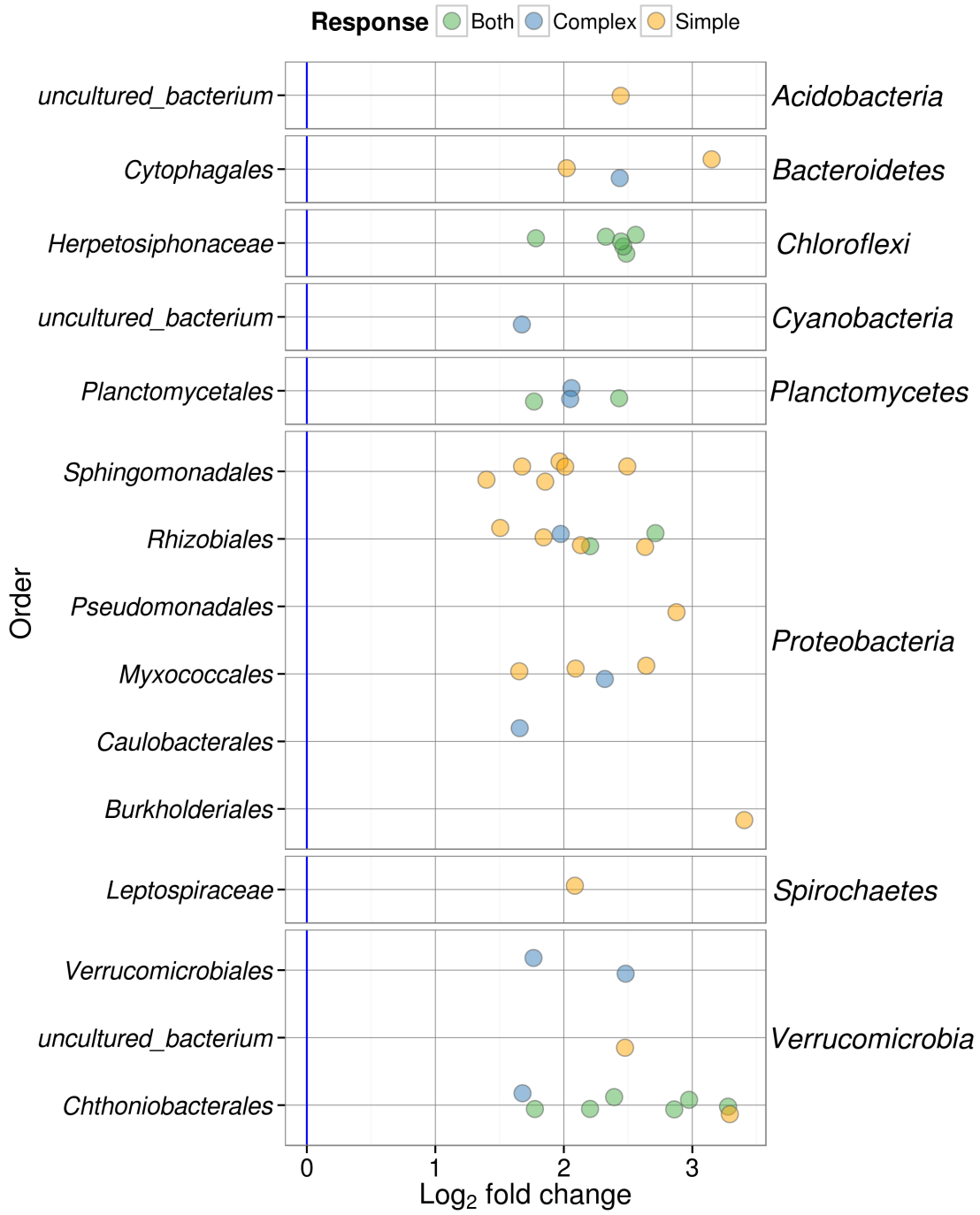

Figure 3.4 Log_2 fold change of responders found in the simple treatment (orange), complex treatment (blue), or both (green). Log_2 fold change value is the magnitude of enrichment for an OTU. Classifications on the right are phylum and left are orders.

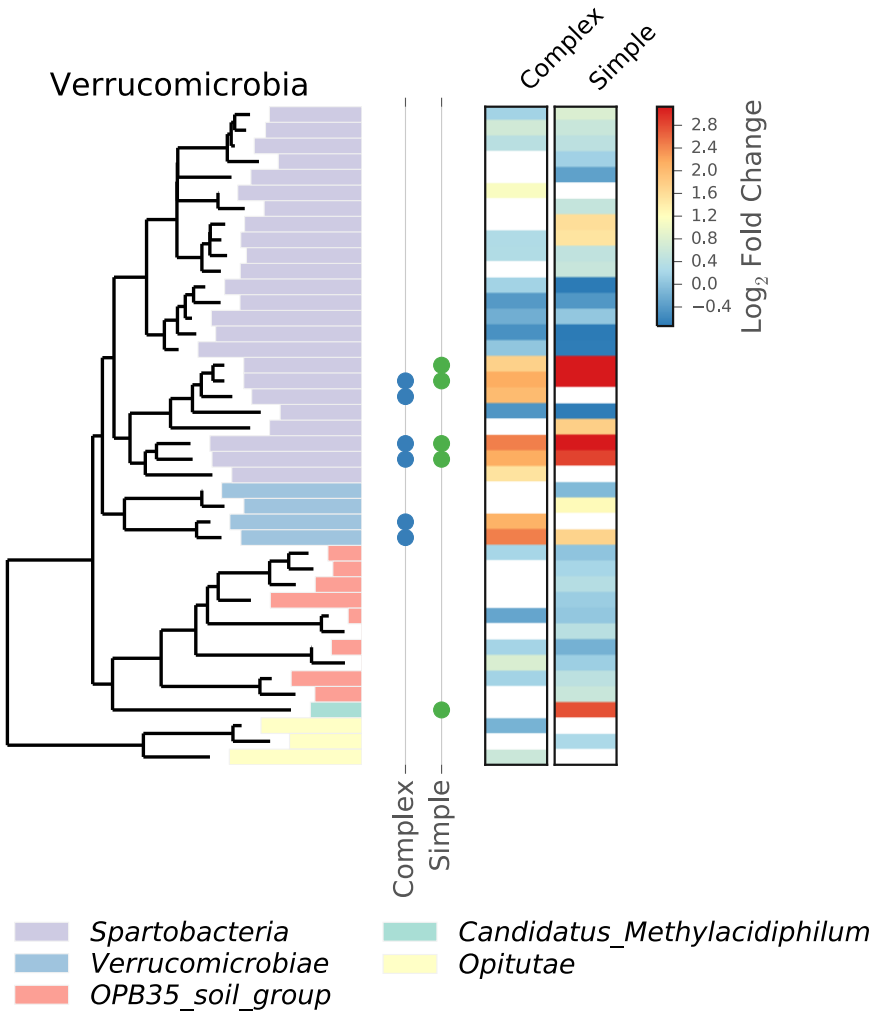

Figure 3.5 *Verrucomicrobia* 16S rRNA gene phylogenetic tree colored by class. Points beside tips signify an OTU that significantly assimilated ^{13}C in the complex treatment (blue) or the simple treatment (green). Heat map indicates $\log_2$ fold change value for each OTU in the complex (left) and simple (right) treatment. Log_2 fold change represents the magnitude of enrichment in the ^{13}C-treatment relative to the control.

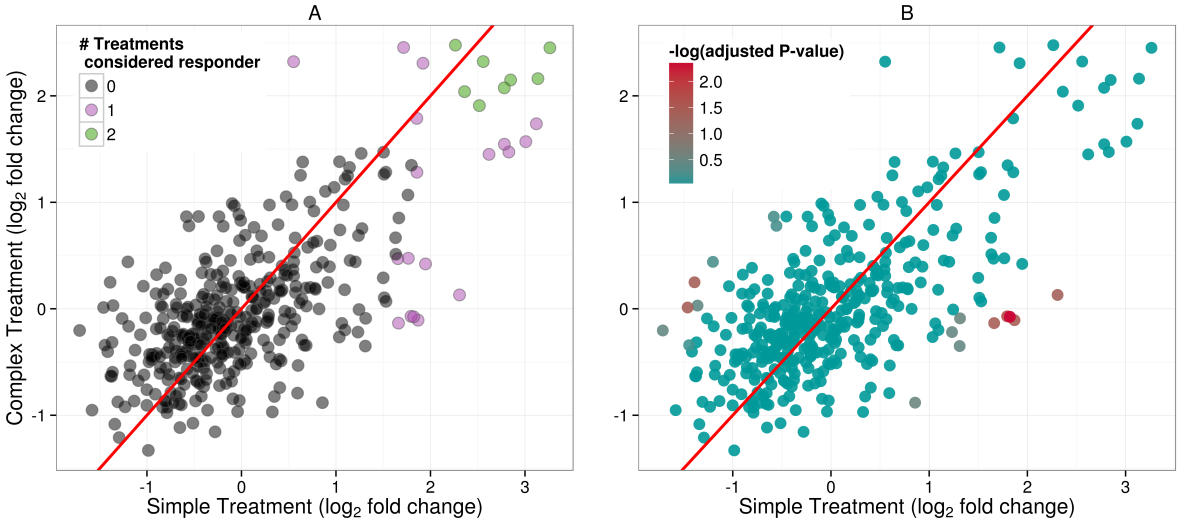

Figure 3.6 Linear regression of log_2 fold change values for all OTUs in both treatments. Log_2 fold change value is the magnitude of enrichment for an OTU. (A) OTUs are colored by the number of treatments it is considered a cellulose responder (0, black; 1, purple; 2, green). Red lines are a 1:1 reference line. OTUs with a significant enrichment in one treatment relative to the other (simple versus complex) are red (B).

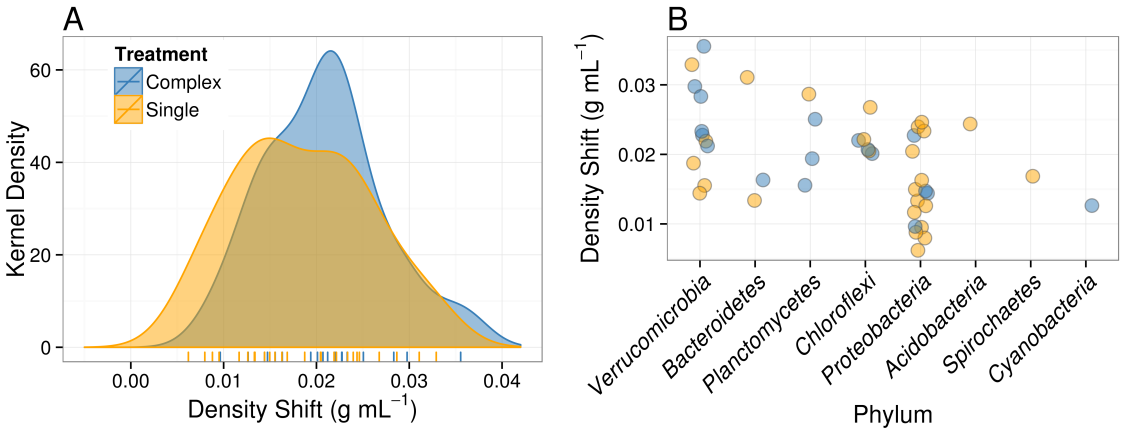

Figure 3.7 Density shifts of all responders for the complex treatment (blue) and simple treatment (orange)(A) and within each phylum (B). There were no significant differences in density shifts of cellulose responders between the two treatments.

3.7 Tables

Table 3.1 Soil carbon and nitrogen before and after incubations

Treatment	Total C	Total N	^{13}C-Cellulose	C:N
Simple (T_0)	13.04	1.16	0.89	11.24
Complex (T_0)	15.15	1.45	0.89	10.45
Simple (T_f)	14.36	1.54	0.25	9.32
Complex (T_f)	14.70	1.65	0.35	8.91

Note: Values presented in this table represent only the ^{13}C-series for each treatment (simple or complex). Separate values are presented in this table at the start (T_0) and end (T_f) of the experiment. All values are in units of mgC or mgN g^{-1} dry soil. Total C and N values include the C and N of the amendments added.

Table 3.2: ^{13}C-cellulose responders in the simple treatment

OTU ID	**Fold change** [a]	**Top BLAST hits** [b]	**BLAST %ID** [b]	**Phylum;Class;Order** [c]
OTU.569	2.15	No hits of at least 95% identity	84.16	*Acidobacteria Candidatus-Solibacter uncultured-bacterium*
OTU.382	2.98	No hits of at least 95% identity	89.19	*Bacteroidetes Cytophagia Cytophagales*
OTU.525	1.9	*Cytophaga hutchinsonii ATCC 33406*	98.63	*Bacteroidetes Cytophagia Cytophagales*
OTU.64	2.78	No hits of at least 95% identity	89.5	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.98	2.56	No hits of at least 95% identity	88.18	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.4322	2.26	No hits of at least 95% identity	89.14	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.285	2.52	No hits of at least 95% identity	90.87	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.766	2.36	*Devosia insulae*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.206	2.31	*Anderseniella baltica*	95.89	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.73	1.95	*Mesorhizobium temperatum*, *Mesorhizobium caraganae*, *Mesorhizobium robiniae*, *Mesorhizobium gobiense*, *Mesorhizobium sp. Ala-3*, *Mesorhizobium tarimense*, *Mesorhizobium tianshanense*, *Mesorhizobium metallidurans*, *Mesorhizobium mediterraneum*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.19	1.86	*Rhizobium alamii*, *Rhizobium mesosinicum*, *Rhizobium mongolense*, *Arthrobacter viscosus*, *Rhizobium sullae*, *Rhizobium yanglingense*, *Rhizobium loessense*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.263	1.77	No hits of at least 95% identity	94.06	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.89	2.62	*Sphingomonas trueperi*, *Sphingomonas sp.*, *Sphingomonas pituitosa*, *Caulobacter leidyia*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.1414	1.87	*Sphingomonas kaistensis*	97.72	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.38	1.82	*Kaistobacter terrae*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.17	1.79	*Sphingomonas sp. 382*	97.72	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.20	1.66	*Sphingomonas jaspsi*	98.17	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.2294	1.65	*Kaistobacter sp. Gsoil 634*	97.26	*Proteobacteria Alphaproteobacteria Sphingomonadales*

Table 3.2 – continued from previous page

OTU ID	Fold change	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.114	3.01	*Herbaspirillum sp. SUEMI03*, *Herbaspirillum sp. SUEMI10*, *Oxalicibacterium solurbis*, *Herminiimonas fonticola*, *Oxalicibacterium horti*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5680	2.83	No hits of at least 95% identity	90.05	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.169	2.39	No hits of at least 95% identity	92.27	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.442	1.85	No hits of at least 95% identity	92.24	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.6	2.78	*Cellvibrio fulvus*	100.0	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.945	1.71	*Turneriella parva*	99.54	*Spirochaetes Spirochaetales Leptospiraceae*
OTU.400	2.76	No hits of at least 95% identity	83.64	*Verrucomicrobia Candidatus-Methylacidiphilum uncultured-bacterium*
OTU.185	3.26	No hits of at least 95% identity	85.14	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.266	3.14	No hits of at least 95% identity	83.64	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.2192	3.12	No hits of at least 95% identity	83.56	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.541	2.85	No hits of at least 95% identity	84.23	*Verrucomicrobia Spartobacteria Chthoniobacterales*

[a] Maximum observed log_2 of fold change.
[b] Against Living Tree Project database.
[c] Annotation from Silva database assigned during OTU binning (see methods).

Table 3.3: ^{13}C-cellulose responders in the complex treatment

OTU ID	**Fold change** [a]	**Top BLAST hits** [b]	**BLAST %ID** [b]	**Phylum;Class;Order** [c]
OTU.465	2.32	No hits of at least 95% identity	92.73	*Bacteroidetes Cytophagia Cytophagales*
OTU.4322	2.48	No hits of at least 95% identity	89.14	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.98	2.32	No hits of at least 95% identity	88.18	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.64	2.08	No hits of at least 95% identity	89.5	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.120	1.92	No hits of at least 95% identity	94.52	*Cyanobacteria SM1D11 uncultured-bacterium*
OTU.204	2.31	No hits of at least 95% identity	nan	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.484	2.15	No hits of at least 95% identity	89.09	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.285	1.91	No hits of at least 95% identity	90.87	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.119	2.0	*Brevundimonas alba*	100.0	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.766	2.04	*Devosia insulae*	99.54	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1087	1.9	*Devosia soli*, *Devosia crocina*, *Devosia riboflavina*	99.09	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.633	1.95	No hits of at least 95% identity	89.5	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.185	2.45	No hits of at least 95% identity	85.14	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.266	2.16	No hits of at least 95% identity	83.64	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.541	2.15	No hits of at least 95% identity	84.23	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1023	2.0	No hits of at least 95% identity	80.54	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.83	2.45	*Luteolibacter sp. CCTCC AB 2010415*	97.72	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.638	2.09	No hits of at least 95% identity	93.61	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*

[a] Maximum observed log_2 of fold change.
[b] Against Living Tree Project database.
[c] Annotation from Silva database assigned during OTU binning (see methods).

4 Chapter 4: Timing of root exudate additions affect cellulose degrader composition and activity but not priming of cellulose

4.1 Abstract

Root exudates alter soil organic matter (SOM) decomposition, which could have large impacts on soil carbon (C) retention and global C predictions. In this study we examine the effects of root exudates on the priming of SOM. We amended soil microcosms with ^{13}C-cellulose as a proxy for SOM in the presence or absence of an artificial root exudate solution composed of low molecular weight compounds. Soils receiving the root exudate (RE) were given either one large dose or multiple, small repeat doses of RE. The addition of RE did not cause any priming of cellulose decomposition. Furthermore, cellulose decomposition dynamics did not vary between treatments. We identified 1,000 OTUs that assimilated ^{13}C from cellulose and they varied between treatments and over time.

4.2 Introduction

Soils receive pulse inputs from fertilizers, organic substrates secreted by roots, or plant residues (Blagodatsky et al., 2010), which induce a cascade of belowground reactions (Blagodatskaya et al., 2014). The short-term changes in soil organic matter (SOM) turnover resulting from the addition of organic and/or inorganic substrates are termed 'priming effects' (PE)(Kuzyakov et al., 2000). Plant roots influence surrounding soils chemically, physically, and biologically (Somers et al., 2004). This zone of influence is known as the rhizosphere. Plant-microbe

interactions in the soil govern nutrient cycling processes (Singh et al., 2004; Bais et al., 2006).

Plant roots release a range of compounds in the form of exudates, lysates, mucilage, secretions, and dead cell material (Somers et al., 2004; Lynch 1990; Prosser et al., 2006). The addition of nutrients triggers dormant soil microorganisms into activation (Blagodatskaya, Kuzyakov 2008; De Nobili et al., 2001; Kuzyakov, Bol 2006), and in turn, the rhizosphere microbial community transforms in size, structure, and activity (Somers et al., 2004; Blagodatsky et al., 2010; Griffiths et al., 2008; Paterson et al., 2009; De Deyn et al., 2008; de Graaff et al., 2010). The response of the soil microbial activity to altered amounts and availability of C results in changes to SOM turnover (Blagodatskaya, Kuzyakov 2008). Long-term consequences of decomposition on SOM depend on the membership of the active decomposers in the microbial community (Wutzler, Reichstein 2008). This cascade of events could have strong implications for soil C storage.

Priming effects can be positive or negative and vary in magnitude. The addition of readily available substrates can result in large and small, positive and negative, or no PE at all (Hamer, Marschner 2002; Hamer, Marschner 2005; Dalenberg, Jager 1989; Wu et al., 1993; Kuzyakov, Bol 2006; Blagodatskaya et al., 2007; de Graaff et al., 2010). The amount and composition of root exudates vary from plant to plant and with environmental conditions (Griffiths et al., 1998; Whipps 1990) and these variations determine the magnitude and type of PE (Blagodatskaya, Kuzyakov 2008). The decomposition of readily available C led to a greater PE than the addition of substrates with lower availability such as plant

residues (Conde et al., 2005; Hamer, Marschner 2005; Blagodatskaya, Kuzyakov 2008).

Positive priming was observed when the added C represented less than 15% of microbial biomass C (Blagodatskaya, Kuzyakov 2008). When added C represented greater than 50% of microbial biomass C negative priming was observed (Blagodatskaya, Kuzyakov 2008). Amount of added N also determines the type of priming (Blagodatskaya, Kuzyakov 2008). Generally, PE decreases when substrates are added with N and increases when substrates are added without N (Blagodatskaya, Kuzyakov 2008). However, PE has been observed when substrates are added with N at low amounts relative to the C content of the amendment (Conde et al., 2005; Hamer, Marschner 2005). The conflicting evidence of the effects of N on PE led Blagodatskaya and Kuzyakov (2008) to propose that the effect of N on PE is related to the C:N ratio, where a high C:N results in positive priming. In other words, positive priming is proposed to occur as a result of the microbial community mining SOM for N when nutrient inputs are N limited. When nutrient additions are not N limited, the microbial community preferentially metabolizes the nutrient inputs rather than SOM.

The differences in PE are accompanied by changes in microbial community structure (Bell et al., 2003; Blagodatskaya et al., 2007; Falchini et al., 2003; Kramer, Gleixner 2006; Landi et al., 2006; Blagodatskaya, Kuzyakov 2008), which suggests community structure mediates the priming effect. Understanding the causes of this variation will facilitate our understanding of root impacts on the soil C cycle. Since root exudates directly impact the microbial community, which in turn, impact the

magnitude and direction of SOM dynamics – it stands to reason that understanding how the microbial community changes when provided with root exudates will reveal soil C dynamics.

To shed light on the impact of root exudation on SOM decomposition, we examined how labile C compounds, commonly found in root exudates, alter plant residue decomposition and the bacterial taxa responsible for those changes. This is achieved by measuring the use of ^{13}C-cellulose in soil microcosms amended with artificial root exudates added at different rates. The artificial root exudate contained an average of compounds commonly found in root exudates and combined in realistic ratios (Kraffczyk et al., 1984; Baudoin et al., 2003; Bürgmann et al., 2005). We added an artificial root exudate cocktail at two concentrations (700 μg C g^{-1} soil or 100 μg C g^{-1} soil) with ^{13}C-labeled or unlabeled (^{12}C) cellulose and measured respiration over 45 days. The high dose treatment was added as a single addition at the beginning of the incubations while the low dose treatment (100 μg C g^{-1} soil) was added once a week for 7 weeks, until the total root exudate C added was the same as the high dose treatment (700 μg C g^{-1} soil). The goal was to contrast the effects cellulose use based on the timing of root exudate additions. Another treatment received ^{13}C-labeled or unlabeled cellulose without any artificial root exudate. These treatments enabled us to determine cellulose-specific respiration resulting from the various amendments. The decomposition of ^{13}C-cellulose was monitored by measuring ^{13}C-CO_2 efflux over 45 days. Cellulose responders were identified for each treatment using SIP gradient centrifugation, fractionation, and next generation sequencing. We predict that cellulose degraders will

phylogenetically vary based on the addition and timing of root exudate. However, we predict that this variation will not result in a difference in the amount of cellulose degraded at the end of the incubation.

4.3 Methods

4.3.1 Soil collection and preparation

Soils were collected from an organic farm in Penn Yan, New York. These soils are Honoeye/Lima, a silty clay loam on calcareous bedrock. Twelve soil cores (5 cm diameter x 10 cm depth) were collected in duplicate from six random sampling locations in a single field by using a slide hammer bulk density sampler (coordinates: (1) N 42° 40.284' W 77° 02.398', (2) N 42° 40.310' W 77° 02.456', (3) N 42° 40.323' W 77° 02.435', (4) N 42° 40.321' W 77° 02.406', (5) N 42° 40.332' W 77° 02.392', (6) N 42° 40.349' W 77° 02.368') on October 25, 2012. Cores were sieved to 2 mm, homogenized, and stored at 4°C (for 1-2 weeks). Carbon and nitrogen content of homogenized soils were 11.88 ± 0.97 (s.d.) mg C g^{-1} dry soil and 1.24 ± 0.1 (s.d.) mg N g^{-1} dry soil.

4.3.2 Cellulose production

Bacterial cellulose (both ^{12}C and ^{13}C) was produced by *Gluconoacetobacter xylinus* grown in Heo and Son (Heo, Son 2002) liquid minimal medium made with 0.1% glucose. Cellulose was produced in 1 L Erlenmeyer flask containing 100 mL Heo and Son minimal medium that were inoculated with three colonies of *G.xylinus* grown on Heo and Son 0.1% glucose agar plates without inositol at 30°C. Flasks were incubated statically in the dark at 30°C for 2-3 weeks until a thick cellulose

pellicule had formed. Cellulose pellicules were collected and autoclaved for 30 min with two volumes 1% Alconox. Cellulose pellicules were rinsed repeatedly with deionized water then purified by dialysis in 1 L deionized water for 12 hrs. Dialysis was repeated 10 times. Pellicules were then dried overnight (60°C), cut into pieces, and ground to 53 μm – 250 μm using 5100 Mixer/Mill (SPEX SamplePrep, Metuchen, NJ) and dry sieved. The particulate size range was selected to be representative of particulate organic matter in soils (Cambardella, Elliott 1992).

4.3.3 Soil microcosms

Preincubation

An aliquot of soil was dried at 105°C overnight to determine soil moisture content gravimetrically. Microcosms (70 total) were created by adding the equivalent of 10g dry soil weight (of the sieved soil) to a 250 mL Erlenmeyer flask capped with a butyl rubber stopper. Microcosm headspaces were flushed with air every 3 days. Microcosms were pre-incubated at 25°C for 2 weeks until the soil respiration rate had stabilized. Sieving causes a transient increase in soil respiration rate presumably due to the liberation of fresh labile soil organic matter (Datta et al., 2014). Pre-incubation ensures that this labile organic matter is consumed and/or stabilized prior to the beginning of the experiment.

Artificial root exudate composition

The artificial root exudate was modeled after a range of compounds reported to occur in root exudates (Kraffczyk et al., 1984; Baudoin et al., 2003; Bürgmann et al., 2005); by mass, 80% sugar (46.5% glucose, 15.4% fructose, 18.1% sucrose),

10% organic acids (3% succinate, 4.5% citrate, 2.5% malate), and 10% amino acids (4.5% glutamate, 3.8% alanine, 1.7% serine). The 700 μg C g^{-1} soil pulse treatment stock solution contained: D-glucose (32.6 mg mL^{-1}), D-fructose (10.8 mg mL^{-1}), D-sucrose (12.05 mg mL^{-1}), succinic acid (2.06 mg mL^{-1}), citric acid (3.35 mg mL^{-1}), malic acid (1.97 mg mL^{-1}), glutamic acid (3.96 mg mL^{-1}), alanine (2.63 mg mL^{-1}), and serine (1.37 mg mL^{-1}). The 100 μg C g^{-1} soil continuous treatment stock solution contained: D-glucose (4.65 mg mL^{-1}), D-fructose (1.54 mg mL^{-1}), D-sucrose (1.72 mg mL^{-1}), succinic acid (0.29 mg mL^{-1}), citric acid (0.50 mg mL^{-1}), malic acid (0.28 mg mL^{-1}), glutamic acid (0.57 mg mL^{-1}), alanine (0.38 mg mL^{-1}), and serine (0.20 mg mL^{-1}).

Treatments

Four treatments all receiving the same volume (250 μL) of aqueous additions weekly are described as follows. There are ten microcosms for each unlabeled treatment (^{12}C); 2 replicates per time point (n = 5). The water only treatment was the same except it had 5 replicates for the final time point, resulting in 13 total microcosms. Each ^{13}C-labeled treatment had nine total microcosms - 2 replicates for two time points (days 14 and 28) and 5 replicates on day 45. All treatments that received cellulose were amended with 2 mg cellulose g^{-1} d.w. soil.

Treatment 1: water

A series of microcosm soils in this treatment received no substrate amendments. Weekly aqueous additions consisted of only sterile, deionized water.

Treatment 2: cellulose only

Two series of microcosm soils were set up where one set received ^{13}C-cellulose and the other received ^{12}C-cellulose. Weekly aqueous additions consisted of only water.

Treatment 3: root exudate pulse

Soils were dosed with a single, large dose of root exudate (described above) one week before cellulose addition to 'prime' the microbial community for cellulose decomposition. Microcosms were split into two series where one set of microcosms received ^{13}C-cellulose and the other set received ^{12}C-cellulose. Aqueous additions added with cellulose and every week thereafter consisted of only water.

Treatment 4: root exudate repeated

Soils were dosed with a single, small dose of root exudate (described above) one week before cellulose addition to 'prime' the microbial community for cellulose decomposition. Microcosms were split into two series where one set of microcosms received ^{13}C-cellulose and the other set received ^{12}C-cellulose. Aqueous additions added with cellulose and every week thereafter consisted of the low dose root exudate.

Amendment additions and incubation

The volume of the liquid addition was chosen to achieve 50% water holding capacity of the soil. Water holding capacity of 50% was chosen to achieve ~70% water filled pore space in these soils based on soil texture, which is the optimal water content for respiration (Linn, Doran 1984). Each microcosm received a

'priming' dose (250 μL) of either only water, a single pulse of 700 μg C (g^{-1} soil) artificial root exudate, or 100 μg C (g^{-1} soil) artificial root exudate one week before cellulose addition (described above). One week after 'priming' dose was added, cellulose (2 mg g^{-1} soil) was evenly distributed as a dry addition. Each week after the initial 'priming' dose, a liquid addition (0.25 mL) of either water (water only and root exudate pulse treatment) or 100 μg C (g^{-1} soil) artificial root exudate (repeated root exudate treatment) was added to the microcosms.

Microcosms were incubated in the dark at ambient room temperature. The headspace was sampled and measured via gas chromatograph mass spectrometry (GCMS) throughout the incubations for CO_2 respiration, beginning at 'priming' dose addition. Microcosm stoppers were removed every three days and flushed with air to prevent anoxia.

Replicate microcosms were sampled destructively and stored at -80°C (until nucleic acid processing) on the same day as weekly additions; microcosms are not given an amendment addition the day they are harvested. Replicate microcosms were harvested at days 1 (same day as cellulose addition, only unlabeled treatments harvested), 7 (only unlabeled treatments harvested), 14, 28, and 45 (all treatments).

Gas chromatography

Periodically, CO_2 efflux was measured via GCMS using a Shimadzu QP2010S GCMS plumbed with Carboxen-1010 PLOT column (G001075, Supelco, St. Louis, MO). Microcosm headspace samples were injected into the GC at an injection port temperature of 200°C. The oven temperature was held at 30°C for 7.5 min then

ramped to 230°C at a rate of 24°C min^{-1}. The column flow proceeded at a rate of 1.59 mL min^{-1}. At the GC-MS interface the temperature was 230°C. The quadrupole MS scanned for m/z 44, 45, and a total ion count (TIC).

Soil isotope submission

An aliquot of microcosm soil from each treatment was isotopically analyzed at Cornell University Stable Isotope Laboratory to determine amount of ^{13}C that remained at the end of the experiment.

4.3.4 Nucleic acid extraction

Nucleic acids were extracted from 0.25 g soil using a modified Griffiths protocol (Griffiths et al., 2000). Cell lysis was performed by bead beating for 1 min at 5.5 m s^{-1} in 2 mL lysis tubes containing 0.5 g of 0.1 mm diameter silica/zirconia beads (treated at 300°C for 4 hours to remove RNases), 0.5 mL extraction buffer (240 mM phosphate buffer 0.5% N-lauryl sarcosine), and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1) for 1 min at 5.5 m s^{-1}. After lysis, 85 µL 5 M NaCl and 60 µL 10% hexadecyltriammonium bromide (CTAB)/0.7 M NaCl were added to lysis tube, vortexed, chilled for 1 min on ice, and centrifuged at 16,000 x g for 5 min at 4°C. The aqueous layer was transferred to a new tube and reserved on ice. To increase DNA recovery, the pellet was back extracted with 85 µL 5 M NaCl and 0.5 mL extraction buffer. The aqueous extract was washed with 0.5 mL chloroform:isoamyl alcohol (24:1). Nucleic acids were precipitated by addition of 2 volumes polyethylene glycol solution (30% PEG 8000, 1.6 M NaCl) on ice for 2 hrs, followed by centrifugation at 16,000 x g, 4°C for 30 min. The supernatant was

discarded and pellets were washed with 1 mL ice cold 70% EtOH. Pellets were air dried, resuspended in 50 μL TE and stored at -20°C. Each DNA extraction was further purified using Illustra Microspin G50 columns (GE Healthcare, Piscataway, NJ; 27-5330-01) per manufacturers protocol with the longer spin options. This extra purification step was to remove excessive humic substances that remained after the DNA extraction process.

To prepare nucleic acid extracts for isopycnic centrifugation, DNA was size selected (>4 kb) with the Blue Pippen (Sage Science, Beverly, MA) using the 0.75% agarose gel cassette (Sage Science, BLF7510). Cassettes and samples were prepped per manufacturers protocol with one well dedicated to an external standard (Marker S1). Samples were ran using pulse field voltage and size selection for 4,000-14,000 kb. Size selected DNA was eluted into a collection well containing a mixture of running buffer and DNA. Eluted DNA was transferred to a microfuge tube. To increase DNA recovery, 40 μL 0.1% tween (from cassette kit) was added to the collection well, incubated for 1 min, and transferred to the same microfuge tube containing eluted DNA and stored at -20°C.

4.3.5 Isopycnic centrifugation and fractionation

For days 14, 28, and 45, isopycnic gradients were setup using a modified protocol from Neufeld et al.(2007); three gradients for each of six treatments and one additional gradient for a biological replicate of the continuous treatment at day 28. A cesium chloride (CsCl) density gradient solution of an average density 1.69 g mL^{-1} was used to separate ^{13}C-labeled and unlabeled (^{12}C) DNA. The gradient buffer

(pH 8.0) used for the density gradient solution was composed of 15 mM Tris-HCl, 15 mM EDTA, 15 mM KCl. Each centrifuge tube was loaded with the CsCl density gradient solution and approximately 5 μg of DNA, then centrifuged on a Beckman Coulter Optima™ MAX-E ultracentrifuge using a TLA-110 fixed-angle rotor for 66 h at 55,000 rpm and room temperature (RT).

Fractions of ~100 μL were collected from below by displacing the DNA-CsCl-gradient buffer solution in the centrifugation tube with water using a syringe pump at a flow rate of 3.3 μL s^{-1} (Manefield et al., 2002) into a deep well 96-well plate (Corning, Tewksbury, MA; P-96-450V-C-S). The refractive index (R_i) of each fraction was measured using a Reichart AR200 digital refractometer modified as previously described (Buckley et al., 2007) to measure a volume of 5 μL. The R_i was corrected to account for the R_i of the gradient buffer using the equation $[R_{i\ corrected}] = [R_{i\ observed}] - ([R_{i\ buffer}] - 1.3333)$. Then the buoyant density was calculated from the $[R_{i\ corrected}]$ using the equation $\rho = a\eta - b$, where ρ is the density of the CsCl (g ml^{-1}), η is the $[R_{i\ corrected}]$, and *a* and *b* are coefficient values of 10.9276 and 13.593, respectively, for CsCl at 20°C (Birnie 1978).

The collected DNA fractions were purified using Agencourt AMPure XP purification (Beckman Coulter, Brea, CA; A63880). In brief, 180 μL of AMPure XP solution was added to each DNA fraction and incubated for 15 min at room temperature. The 96 well plate containing the DNA fraction and AMPure XP mixture was placed on a magnetic plate until solution cleared (~10 min). Solution was aspirated from each well, and washed three times with ice cold 70% EtOH. The 96 well plate was removed from the magnet, 50 μL TE was added to each well, and

incubated for 2 min. The 96 well plate was placed on the magnetic plate, incubated for 5 minutes, and TE containing DNA was eluted to a new 96 well plate.

Purified DNA fractions were quantified using Quant-IT PicoGreen dsDNA assay (Life Technologies, Grand Island, NY; P7589) and FilterMax F5 plate reader (Molecular Devices, Sunnyvale, CA). For every gradient, *ca.* 20 fractions were chosen for sequencing between the density range 1.67-1.75 g mL^{-1} as previously described (this dissertation, Chapter 2).

4.3.6 DNA sequencing

A total of 19 gradients (395 fractions) and 71 bulk DNA samples were amplified for paired-end-read Illumina MiSeq sequencing (n = 465).

Primers and PCR

Dual barcoded Illumina primers were designed as described in Kozich *et al.* (2013) for paired-end-read assembly. In brief, primers included the appropriate Illumina adapter, an 8 nt barcode index, a 10 nt pad sequence, a 2 nt linker, and a primer sequence for the V4/V5 region of the 16S rRNA gene. The forward adapter sequence is 5'-AATGATACGGCGACCACCGAGATCTACAC-3' and the 515F primer sequence is 5'-GTGYCAGCMGCMGCGGTRA-3'. The reverse adapter sequence is 5'-CAAGCAGAAGACGGCATACGAGAT-3' and the 927R primer sequence is 5'-CCGYCCAATTYMTTTRAGTTT-3'.

Each sample (in triplicate) was PCR amplified in a 25 µL reaction using 12.5 µL Q5 high fidelity 2X master mix (New England BioLabs, Ipswich, MA; M0492), 2.5 µL 10 µM 927R primer, 2.5 µL 10 µM 515F primer, 0.625 µL 50-fold diluted Pico

green (Life Technologies, Grand Island, NY; P7589), 9.4 μL DNA template (5 ng) and H_2O. The PCR conditions were as follows: initial denaturation at 98°C for 30 sec, followed by 30 cycles of denaturation at 98°C for 5 sec, annealing at 50°C for 30 sec, and extension at 72°C for 10 sec. A final extension at 72°C for 2 min completed the amplification.

Sequencing

Samples were normalized using SequalPrep™ normalization plates (Invitrogen, Carlsbad, CA; A10510), then pooled in equimolar concentrations. Three amplicon pools of *ca.*125 samples, one pool with 70 samples, and one pool of *ca.*30 samples were sequenced on MiSeq (2 x 300 bp, Illumina) system at Cornell University CORE facilities (Ithaca, NY). Each pool (n = 5) was submitted at 5 ng μL^{-1} and with 100 μM read1 (5'-AATGTTTTAATGGTGYCAGCMGCMGCGGTRA-3'), 100 μM read2 (5'-CAACCCAACAGGCCGYCCAATTYMTTTRAGTTT-3'), and 100 μM index sequence (reverse complement of read2).

4.3.7 Post sequencing analysis

Sequence quality control

Forward and reverse reads were assembled using PEAR (v0.9.2, Zhang et al., 2014). There were 82,531,119 reads assembled (95%) out of 86,661,821 total reads for all five MiSeq runs. Assembled and demultiplexed reads were initially screened by maximum expected errors at a specific read length threshold (Edgar, 2013). Specifically, any read that exceeded a maximum expected error threshold of 1.0 was removed. Any reads with an ambiguous base (N) were removed from the data set.

Then, sequences were aligned to the Silva SSU rRNA database (release 119, Pruesse et al., 2007) using the Mothur software package (Schloss et al., 2009). Sequences with homopolymers greater than eight were removed from the dataset using Mothur.

Sequence clustering

Remaining reads were clustered into OTUs (≥97%) using USEARCH (Edgar 2010) based on the UPARSE-OTU algorithm (Edgar 2013). Binned OTUs were then taxonomically annotated using the "UClust" taxonomic annotation framework in the QIIME software package (Edgar, 2010; Caporaso et al., 2010) with cluster seeds from Silva SSU rRNA database (Pruesse et al., 2007) with 97% sequence identity OTUs as reference (release 111). Reads annotated as "chloroplast", "Eukaryota", "Archaea", "unassigned" or "mitochondria" were culled from the dataset. Finally, reads were aligned to the Silva reference alignment provided by the Mothur software package (Schloss et al., 2009) using the Mothur NAST aligner (DeSantis et al., 2006). All reads that did not align to the expected amplicon region of the SSU rRNA gene were discarded. After quality control and binning there were 14,934,544 sequences that clustered into 10,361 OTUs.

Phylogenetic analysis

Alignment of OTU centroid SSU rRNA genes was done with SSU-Align, which is based on Infernal (Nawrocki et al., 2009; Nawrocki, Eddy 2013). Columns in the alignment that were not included in the SSU-Align covariance models or were aligned with poor confidence (less than 95% of characters in a position had

posterior probability alignment scores of at least 95%) were masked for phylogenetic reconstruction. Additionally, the alignment was trimmed to coordinates such that all sequences in the alignment began and ended at the same positions. FastTree (Price et al., 2009) was used to reconstruct the phylogeny.

Community sequence analysis

Nonmetric Multidimensional Scaling (NMDS) uses a distance (or dissimilarity) matrix to find the least stressed relationship between samples in a low dimensional space. Specifically, weighted unifrac (Lozupone, Knight 2005) distances were used for NMDS analyses. The Phyloseq (McMurdie, Holmes 2013) wrapper for Vegan (Dixon 2003) (both R packages) was used to compute sample values along the axes. The ordinations presented here are graphical representations of the sample relationships as determined by NMDS analysis. GGplot2 (Wickham 2009) was used to display sample points in the two-dimensional space. In general, samples in close proximity have more similar microbial composition than samples spaced further away. Adonis tests (Anderson 2001) were done with 1000 permutations to compare community compositions.

Identifying OTUs that incorporated ^{13}C into their DNA

We used DESeq2, a RNA-Seq differential expression statistical framework (Love et al., 2014) to find OTUs enriched in heavy fractions of labeled gradients relative to corresponding density fractions in control gradients(Campbell et al. 2015, in progress, McMurdie, Holmes 2014). We use the term "differential abundance", coined by McMurdie *et al.* (2014), to denote OTUs that have different

proportion means across sample classes (in this case the only sample class is labeled:control). CsCl gradient fractions were categorized as “heavy” or “light”. The heavy category denotes fractions with density values between 1.7125-1.755 g mL^{-1}. Since we are only interested in enriched OTUs (labeled versus control), we used a one-sided Wald-test for differential abundance (the null hypothesis is the labeled:control proportion mean ratio for an OTU is less than a selected threshold). P-values were corrected with the Benjamini and Hochberg method (Benjamini, Hochberg 1997).

Only OTUs present in at least 25% of the density fraction libraries (within the 1.7125-1.755 g mL^{-1} density window) were evaluated with DESeq2. DESeq2 was used to calculate the moderated log_2 fold change of labeled:control proportion mean ratios and corresponding standard errors for the Wald test at a threshold of 1.0. Those OTUs that exhibit a statistically significant proportional increase, and pass a false discovery rate of 0.1, in heavy fractions from ^{13}C-labeled samples relative to corresponding controls have increased significantly in buoyant density in response to ^{13}C treatment. OTUs that significantly assimilated ^{13}C into their DNA were identified by BLAST searches that were done with the “blastn” program from BLAST+ toolkit (Camacho et al., 2009) version 2.2.29+. Default parameters were always employed and the BioPython (Cock et al., 2009) BLAST+ wrapper was used to invoke the blastn program. Pandas (McKinney 2012) and dplyr (Wickham, Francois 2014) were used to parse and transform BLAST output tables.

4.3.8 Soil isotope data analysis

Soils from all treatments at day 45 were analyzed for soil C and N (mg) content (Table 4.1). All treatments started (T_0) with the same soils and amount of cellulose-C (0.89mgC g^{-1} d.w. soil). Values for cellulose-C remaining in soil at the end of the experiment for each treatment were calculated by subtracting ^{13}Cmg in the ^{12}C-cellulose series from the ^{13}Cmg in the respective ^{13}C-cellulose series; [$^{13}Cmg_{cellulose}$] = [$^{13}Cmg_{13C\text{-}cellulose\ series}$] – [$^{13}Cmg_{12C\text{-}cellulose\ series}$](Table 4.1). ANOVA tests were done for the mg^{13}C remaining in the soil and for the total C remaining in soil.

4.4 Results

4.4.1 Measured CO_2 production

Efflux of CO_2 was sigmoidal (Figure 4.1). There was a large initial flush of total CO_2 (0.277 ± 0.028 mg C g^{-1} soil) in the pulse treatments that was not observed in the cellulose only (0.023 ± 0.004 mg C g^{-1} soil) or repeated root exudate treatments (0.053 ± 0.001 mg C g^{-1} soil)(Figure 4.1). The $^{13}CO_2$ for the labeled (0.003 ± 0.001 mg^{13}C g^{-1} soil) and unlabeled (0.003 ± 0.0001 mg^{13}C g^{-1} soil) series of the pulse root exudate treatment did not vary; therefore, the large efflux is due to the consumption of the added artificial root exudates but not the ^{13}C-cellulose. Both of the root exudate treatments produced more total CO_2 (pulse, 1.87 ± 0.15 mg C g^{-1} soil; repeated, 1.91 ± 0.04 mg C g^{-1} soil) than the cellulose only treatment (1.25 ± 0.1 mg C g^{-1} soil) and this result was significant (Figure 4.2; ANOVA, pvalue: <0.0008). The difference between total efflux of root exudate treatments and cellulose only

efflux (0.62-0.66 mg C g^{-1} soil) can be explained by consumption of carbon in the exudate treatment (total added root exudate 0.7 mg C g^{-1} soil).

There was no discernable difference in the total amount of $^{13}CO_2$ respired between the ^{13}C-series of each treatment (Figure 4.2). Cellulose respiration (flush of $^{13}CO_2$) occurred in three phases for all three treatments (Figure 4.3). The first phase occurred between 8 and 19 days, the second phase from days *ca.* 19-33, and the third phase from days 34-47. This is arbitrarily estimated by an increase in respiration rate (phase I), followed by a decline (phase II), and then it plateaus (phase III) (Figure 4.3). Peaks in the rate of ^{13}C respiration for which the labeled and corresponding unlabeled series in a treatment co-occur are not considered ^{13}C-cellulose specific respiration, as observed in the early time points of the root exudate pulse treatment (Figure 4.3). At these early time points, the amount of ^{13}C respired in the RE pulse treatment for both the labeled and unlabeled series represents the natural abundance of ^{13}C (1.1%). This was determined by comparing the expected amount of ^{13}C (i.e. 1.1% of the total C; m/z TIC) respired to the observed amount of ^{13}C respired at the corresponding time point. Therefore, the observed ^{13}C signal is a result of unlabeled substrate metabolism and not from cellulose. The peak rates for each treatment all occur at day 19 and are 0.05 mg^{13}C d^{-1} (cellulose only), 0.04 mg^{13}C d^{-1} (root exudate pulse), and 0.04 mg^{13}C d^{-1} (repeated root exudate).

4.4.2 Carbon pools remaining in soil

There was no difference in total soil C or N content between the treatments at the end of the incubation (Table 4.1). There was *ca.* 59% less ^{13}C from cellulose in

the root exudate treatments (pulse, 0.24 ± 0.04 $mg^{13}C\ g^{-1}$ soil; repeated, 0.24 ± 0.05 $mg^{13}C\ g^{-1}$ soil) than the cellulose only treatment (0.41 ± 0.15 $mg^{13}C\ g^{-1}$ soil)(Table 4.1) and this result was significant (ANOVA, pvalue: 0.01).

4.4.3 Microbial community composition

Within each treatment (including the labeled and unlabeled series), the bulk (unfractionated) microbial community composition of the soils changed significantly with time (Figure 4.4; Adonis, p-value: 0.002) and bulk (unfractionated) microbial community composition between treatments became increasing dissimilar with time (Figure 4.4; Adonis, p-value: <0.05).

4.4.4 Cellulose-C assimilating OTUs

We contrasted the phylogenetic content of heavy buoyant density fractions from nucleic acid gradients of the ^{13}C-treatment and equivalent ^{12}C-control to identify DNA from organisms that had assimilated ^{13}C (Pepe-Ranney et al., 2015). The amplicon sequence composition of heavy gradient fractions (>1.725 g mL^{-1}) in the ^{13}C-treatments varied from corresponding fractions in the ^{12}C-control of the corresponding treatment and this result was significant (Figure 4.5; Adonis test; p-values: <0.001). This result is consistent with the presence of ^{13}C-labeled DNA in heavy gradient fractions.

OTUs that are significantly enriched in the heavy gradient fractions of ^{13}C-treatments relative to corresponding controls are identified as 'responders'. In other words, a 'responder' is an OTU assimilating ^{13}C from cellulose. There were 369 cellulose responders in the cellulose only treatment, 273 in the repeated root

exudate treatment, and 358 in the root exudate pulse treatment (Table 4.2, Table 4.3, Table 4.4). A total of 33%, 6%, and 7% of the identified responders matched type strains (≥97%) in the Living Tree Project database, respectively. The phylogenetic affiliation of cellulose responders varied with time and by treatment (Figure 4.6). Most of the cellulose responders belonged to *Bacteroidetes*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia* (Figure 4.6, Figure 4.7). These phyla in addition to *Chloroflexi* assimilated ^{13}C-cellulose throughout the time series in all treatments (Figure 4.7). Numerous *Bacteroidetes* OTUs had a strong initial response (i.e. enrichment in abundance) to the pulse treatment that declined over time (Figure 4.7, Figure 4.8). While the initial *Bacteroidetes* response in the other treatments had fewer OTUs, a trend of diminishing response with time was still observed (Figure 4.8). In contrast, the number of verrucomicrobial OTUs, and enrichment in abundance, increased with time (Figure 4.9).

There were 152 cellulose responders shared across all three treatments and 122 more responders observed in at least two treatments. OTUs that assimilate ^{13}C from cellulose in all treatments are able to function over a range of root exudate concentrations (0-700 μg C g^{-1} soil). This range of function is prevalent in many *Verrucomicrobia*, *Proteobacteria*, and *Planctomycetes* OTUs (Figure 4.10, Figure 4.11, Figure 4.12). Whereas, OTUs that respond in only two of the three treatments are able to assimilate ^{13}C-cellulose under some common set of conditions for those two treatments. For instance, *Gemmatimonadetes* is a responder in only the repeated root exudate treatment and the cellulose only treatment, *Elusimicrobia* in the cellulose only and root exudate pulse treatments, and *Firmicutes* in both of the root

exudate treatments (Figure 4.10, Figure 4.11, Figure 4.12). There are, however, a multitude of responders that are detected exclusively in one treatment (Table 4.5). The activity of these cellulose responders is unique to a single treatment; when optimal conditions occur these cellulose responders assimilate ^{13}C-cellulose (log_2 fold change) in one treatment and not in the other treatments (Figure 4.10, Figure 4.11, Figure 4.12).

In each treatment, the amplicon composition of ^{13}C-treatment heavy gradient fractions changed significantly with time (Figure 4.5; Adonis, p-values: <0.05) suggesting a different composition of cellulose responders over time. Some phyla responded throughout the time series while others responded only at a single time point. For instance, responder phyla identified only on day 14 belonged to candidate phylum BRC1 (all treatments), *Nitrospirae* (cellulose only), and candidate phylum WS3 (pulse) (Figure 4.7). Several phyla responded only at later time points such as *Fibrobacteres* (all treatments) or *Elusimicrobia* (cellulose only and root exudate pulse) (Figure 4.7).

4.4.5 Cellulose-C assimilated by responder OTUs

We measured the change in the center of mass (deltaCM) from an OTU's density profile between corresponding control and labeled gradients as a metric for ^{13}C assimilation. The center of mass (CM) of DNA increases as its ratio of ^{13}C to ^{12}C increases. Cellulose responders in the repeated root exudate treatment had the greatest deltaCM (0.020 ± 0.012 s.d.), followed by responders in the cellulose only treatment (0.016 ± 0.011 s.d.), and finally responders in the root exudate pulse treatment (0.014 ± 0.012 s.d.) (Figure 4.13). The deltaCM of responders (resulting

from the degree of labeling) for each treatment was significantly different in all pairwise comparisons (Figure 4.13; Wilcox p-values: <0.001).

The amount of ^{13}C assimilated varies within each phylum (Figure 4.13). In *Bacteroidetes* (*B*) and *Proteobacteria* (*P*), cellulose responders in the root exudate pulse treatment had a significantly smaller deltaCM (*B*, 0.012 ± 0.012 s.d.; *P*, 0.013 ± 0.01 s.d.) than the cellulose only (*B*, 0.023 ± 0.014 s.d.; *P*, 0.016 ± 0.010 s.d.) and repeated root exudate (*B*, 0.025 ± 0.015 s.d.; *P*, 0.018 ± 0.011 s.d.) treatments (Figure 4.14; wilcox, p-values: <0.001). However, there was no discernable difference between the deltaCM values for the cellulose only and repeated root exudate treatments. In *Actinobacteria* (*A*) and *Verrucomicrobia* (*V*), the repeated root exudate responders (*A*, 0.029 ± 0.012 s.d.; *V*, 0.026 ± 0.010 s.d.) have significantly greater deltaCM (Wilcox, p-values: <0.01) than the responders in the cellulose only treatment (*A*, 0.011 ± 0.010 s.d.; *V*, 0.017 ± 0.012 s.d.)(Figure 4.14).

4.5 Discussion

We examined soils microcosms amended with ^{13}C-cellulose in the absence or presence of an artificial root exudate (added as a single, large pulse dose or as several, small repeated doses) to determine whether priming the soil community alters the diversity of cellulose responders and the dynamics of cellulose decomposition. The addition of fresh organic matter such as root exudates (RE) are proposed to alter SOM decomposition dynamics (Kuzyakov et al., 2000). The three proposed mechanisms by which priming is expected to change decomposition dynamics is by changes in microbial community size, structure, and activity (Somers et al., 2004; Blagodatsky et al., 2010; Griffiths et al., 2008; Paterson et al., 2009). This

study measured the effects of root exudate addition and timing on soil microbial community composition and associated differences in SOM decomposition, using labeled cellulose as a proxy for SOM. We found differences in the phylogenetic affiliation of cellulose responders depending on type of amendment and time of sampling. However, there was no difference in the amount of cellulose decomposed between the root exudate treatments, despite differences in cellulose responders between the two root exudate treatments.

4.5.1 Priming of cellulose decomposition

The same amount of cellulose was decomposed in all treatments as determined by $^{13}CO_2$ efflux. In other words, we did not observe any priming of cellulose decomposition as a result of root exudate additions. However, soil isotope data of ^{13}C (originating from cellulose) revealed significantly less ^{13}C remaining in soil at the end of the incubation for both root exudates treatments. It is possible that the unaccounted for ^{13}C in the root exudate treatments, could have been respired as $^{13}CH_4$ during methanogenesis, however, methane was not measured.

An accelerated rate of cellulose mineralization has been observed in soils dosed with 'trigger solutions' which contained a combination of glucose, amino acids, and root exudates (De Nobili et al., 2001). Our findings support a higher rate of cellulose decomposition in the presence of root exudates compared to cellulose decomposition in the absence of root exudates (Figure 4.3).

The difference in timing of the root exudate addition between the two treatments did not cause any differences in cellulose decomposition. In contrast, a previous study found that the addition of a 'trigger solution' (described above)

added over several additions caused 3-times more CO_2 efflux than the same solution added as a single addition (De Nobili et al., 2001). The C mineralization dynamics in that study were measure over a period of 24 days or less (De Nobili et al., 2001). True priming is said to occur on a time scale of weeks to months (Blagodatskaya, Kuzyakov 2008), suggesting that dynamics measured on shorter time scales may not hold as much ecological relevance when considering net SOC changes. In this study, we found no difference in amount of cellulose used after 45 days between the two root exudate treatments despite differences in the timing of root exudate additions. Given that the composition of the amendments applied to soils in the different treatments were the same and there was no difference in the amount of cellulose used in the two root exudate treatments, we would conclude that the quantity of RE does not play a dominant role in decomposition dynamics. It is more likely that RE composition plays a larger role in decomposition dynamics (Shi et al., 2011; Huang et al., 2014)

Turnover of microbial biomass-C is posited to be the C source of real priming observed after the addition of 'trigger solutions' in the study by De Nobili et al. (2001). The source of primed-C, whether from biomass-C or non-biomass C, is debated (De Nobili et al., 2001; Dalenberg, Jager 1989; Wu et al., 1993; Blagodatskaya, Kuzyakov 2008). Microbial biomass-C represents approximately 2% of SOC content (Paul, Clark 1989; Blagodatskaya, Kuzyakov 2008). Real PE is said to occur if the amount of primed C is higher than both microbial biomass C and the added C (Blagodatskaya, Kuzyakov 2008). Using this as a basis for assessing priming of SOC (not the ^{13}C-cellulose) in our microcosm soils, the water treatment is the only

treatment that demonstrated positive priming of SOC. Biomass (at 2% of the final C content 12.82 mg C g^{-1} soil) in the water treatment represents 0.25 mg C g^{-1} soil and there was no added C. The amount of C respired was 0.61 mg C g^{-1} soil, 2.4 times more than estimated biomass-C, thus, positive priming of SOC. This was not observed in the other treatments.

The same amount of C remained in the soil for all treatments after 45 days regardless of C addition. Qiao et al. (2014)found that priming does not necessarily result in a loss of soil C. Although positive priming may liberate SOC, C from microbial biomass and fresh organic matter becomes stabilized, resulting in a net zero or net increase of SOC (Qiao et al., 2014). Thus, if SOC priming occurred in our microcosm soils, but was replaced by added C or biomass-C for a net zero effect, we would be unable to detect it. Priming studies have used isotopic signatures to tease apart C-sources of respired C (Blagodatskaya et al., 2014)

4.5.2 Cellulose responder composition and activity

Decomposition of cellulose occurred in three phases (Figure 4.3). The first phase was between days 8-19, the second phase between days 20-33, and the third between days 34-47. The rate of cellulose mineralization was highest in the first phase (around day 19) and decreased with each successive phase thereafter (Figure 4.3). This trend was observed in all three treatments, regardless of differences in amendments and membership of cellulose responders. These findings support the cellulose mineralization dynamics observed when C3-C4 soils and C3 soils were amended with ^{14}C-cellulose (Blagodatskaya et al., 2014)Therefore, the phylogenetic

affiliation of cellulose responders does not dictate the overall dynamics of cellulose decomposition.

Many of the cellulose responders in the root exudate treatments were taxa that were previously observed to respond positively to the addition of sugars and organic acids to soil (Shi et al., 2011)More specifically, many of the similar responders we found, such as *Chlorobi* and WS3, positively responded to malic acid (Shi et al., 2011)*Firmicutes* responded positively to root exudates containing sugars and organic acids while no *Firmicutes* taxa responded positively to sugars without organic acids (Shi et al., 2011). In this study, addition of root exudates selected for cellulose degraders identified as positively responding to the addition of organic acids (in addition to other RE substrates) in Shi et al. (2011). Therefore, organic acids may be important to the PE of SOM

The timing of root exudate additions impacted the amount of cellulose-C incorporated by cellulose responders. Responders in the repeated root exudate treatment incorporated more cellulose-C than responders in the root exudate pulse treatment. Cellulose responders in the repeated root exudate treatment may preferentially use cellulose due to the reduced competition for this substrate relative to the substrates in the root exudate mixture. In the root exudate pulse treatment, competitive pressure from fast-growing microbes is likely not sustained over time minimizing the need of cellulose responders to restrict their substrate use to only cellulose.

4.6 Conclusion

The addition of root exudates did not cause any priming of cellulose. The timing of root exudate additions, whether repeated or a single dose, did not cause a difference in the total amount of cellulose used. However, the two root exudate treatments selected for cellulose responders with different phylogenetic affiliations. The dynamics of cellulose decomposition did not vary despite different cellulose responders and C additions between treatments.

While cellulose responder composition may have little bearing on cellulose decomposition, it may be important to the fate of biomass-C (Kindler et al., 2006). Mineralization of SOM resulting from PE may be counter balanced by the stabilization of fresh organic matter or microbial biomass. In the future, we should consider how different members of the microbial community allocate C in their biomass and the implications it has for becoming stabilized SOM.

4.7 References

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4.8 Figures

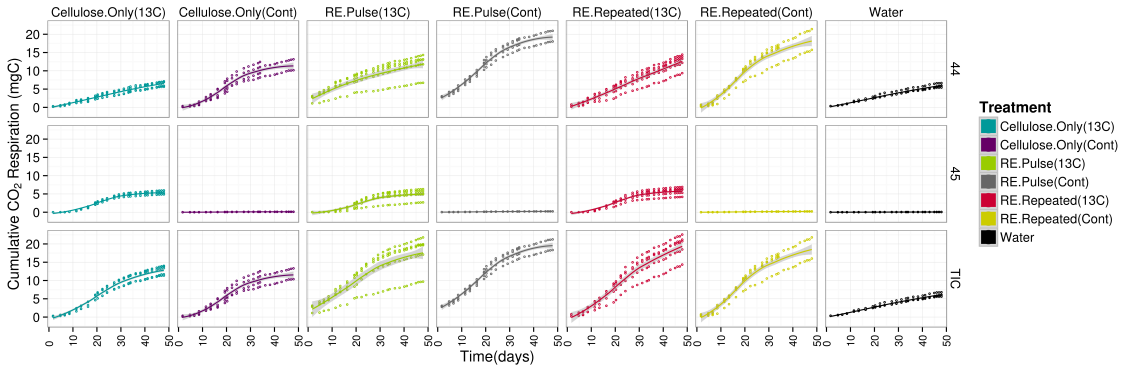

Figure 4.1 Cumulative respiration of $^{12}CO_2$ (*m/z* 44; top row), $^{13}CO_2$ (*m/z* 45; middle row), and both CO_2 isotopes (TIC; bottom row) for each treatment over 48 days. Each treatment has a ^{13}C-series and a ^{12}C-series (cont) representing microcosms amended with ^{13}C-cellulose or ^{12}C-cellulose respectively. $^{13}CO_2$ respiration in the ^{13}C-series of each treatment represents ^{13}C-cellulose specific mineralization.

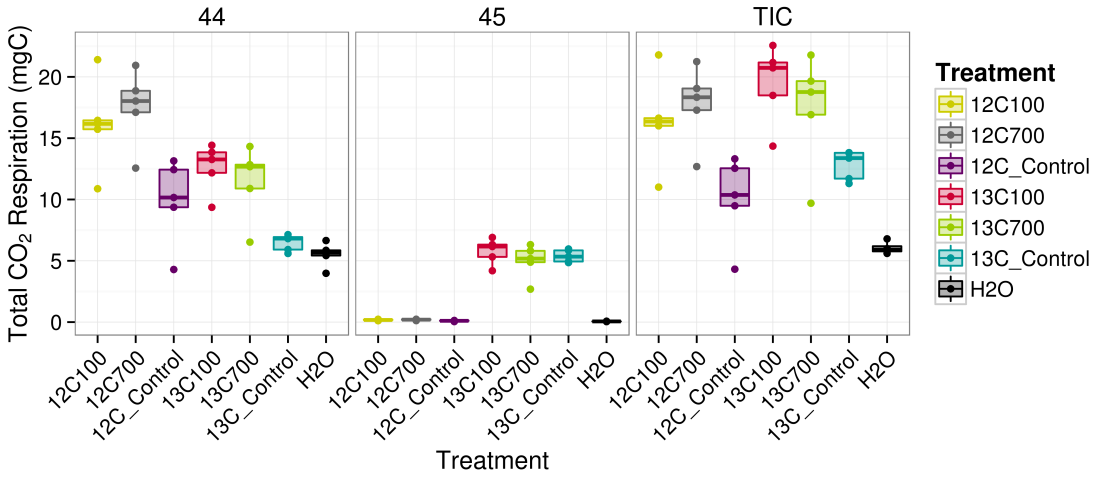

Figure 4.2 Total respiration of $^{12}CO_2$ (*m/z* 44; left), $^{13}CO_2$ (*m/z* 45; middle), and both CO_2 isotopes (TIC; right) for each treatment over 48 days. $^{13}CO_2$ respiration (middle) in the ^{13}C-series of each treatment represents ^{13}C-cellulose specific mineralization. Treatments are cellulose only (^{12}C-control, ^{13}C-control), repeated root exudate (^{12}C100, ^{13}C100), root exudate pulse (^{12}C700, ^{13}C700), and a water only treatment (H_2O). Each treatment has a ^{13}C-series and a ^{12}C-series (cont) representing microcosms amended with ^{13}C-cellulose or ^{12}C-cellulose respectively. Root exudate treatments respired significantly more (ANOVA, p-value: <0.0008) than the cellulose only treatments.

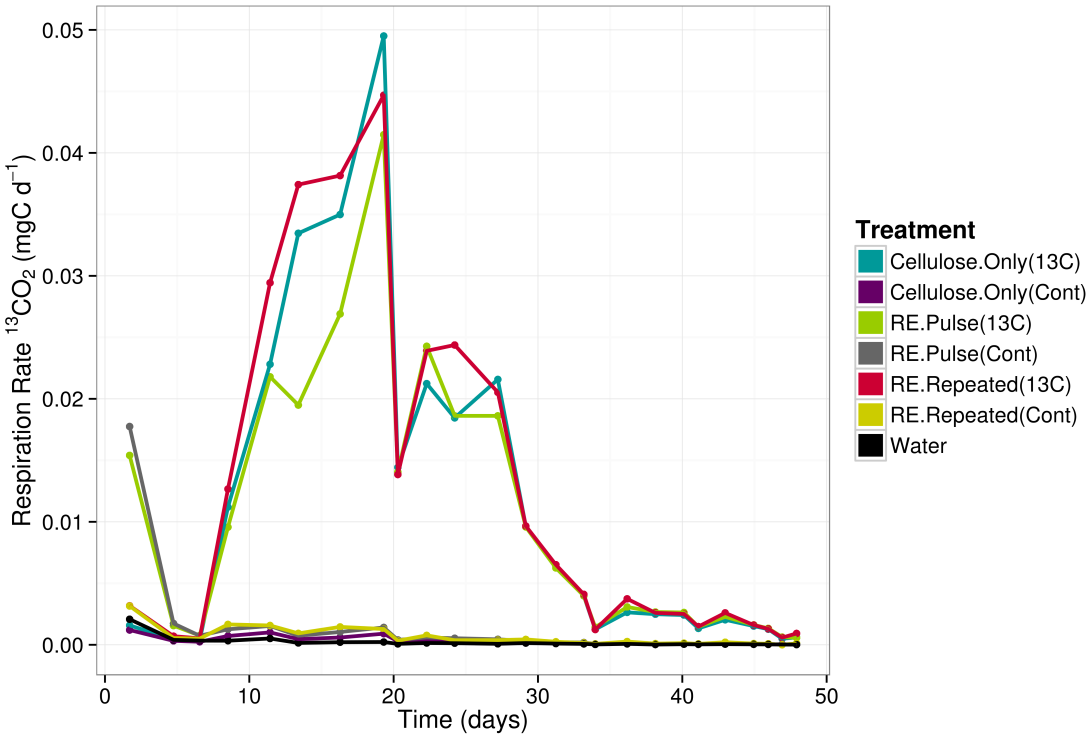

Figure 4.3 Rate of $^{13}CO_2$ respiration for each treatment over 48 days. Each treatment has a ^{13}C-series (13C) and a ^{12}C-series (cont) representing microcosms amended with ^{13}C-cellulose or ^{12}C-cellulose, respectively, except for the water treatment which does not receive any amendments. $^{13}CO_2$ respiration in the ^{13}C-series of each treatment represents ^{13}C-cellulose specific mineralization.

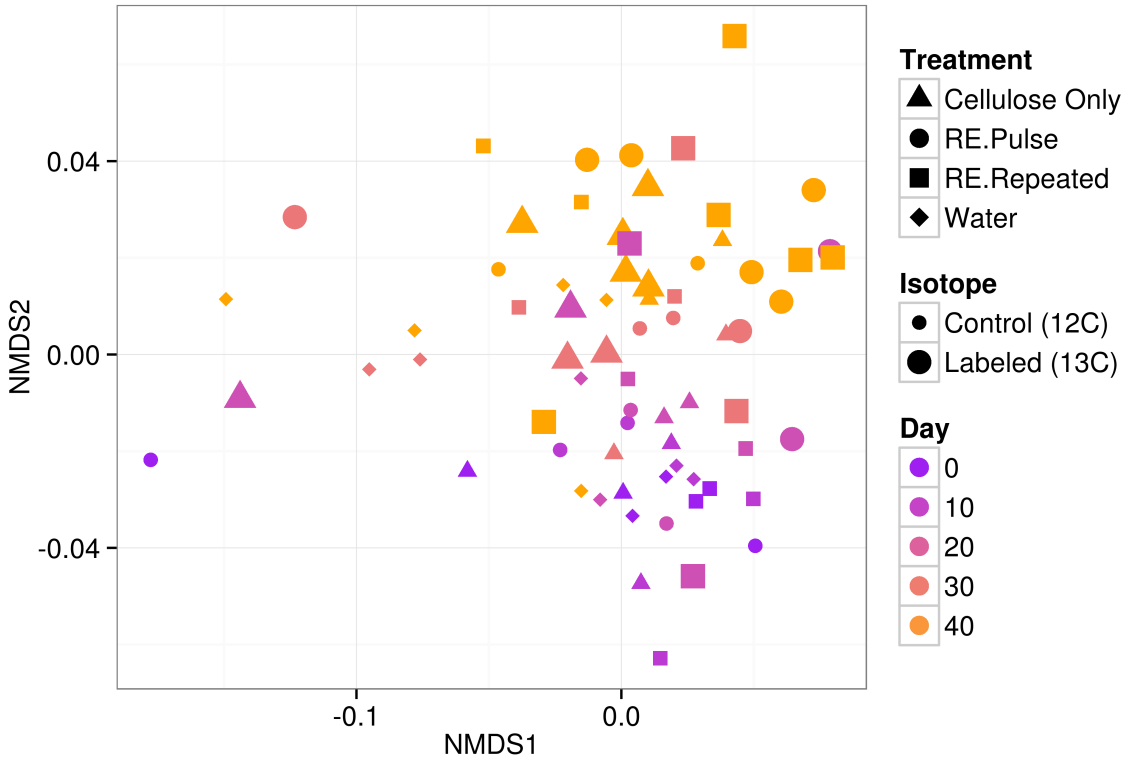

Figure 4.4 NMDS analysis of 16S rRNA gene amplicon composition from bulk soil (unfractionated) DNA for all treatments over time. The point size represents the ^{12}C-series (small) or ^{13}C-series (large) of each treatment. The color represents the day the sample was harvested. Proximity of one sample to another represents the similarity (or dissimilarity) of the community compositions between those samples.

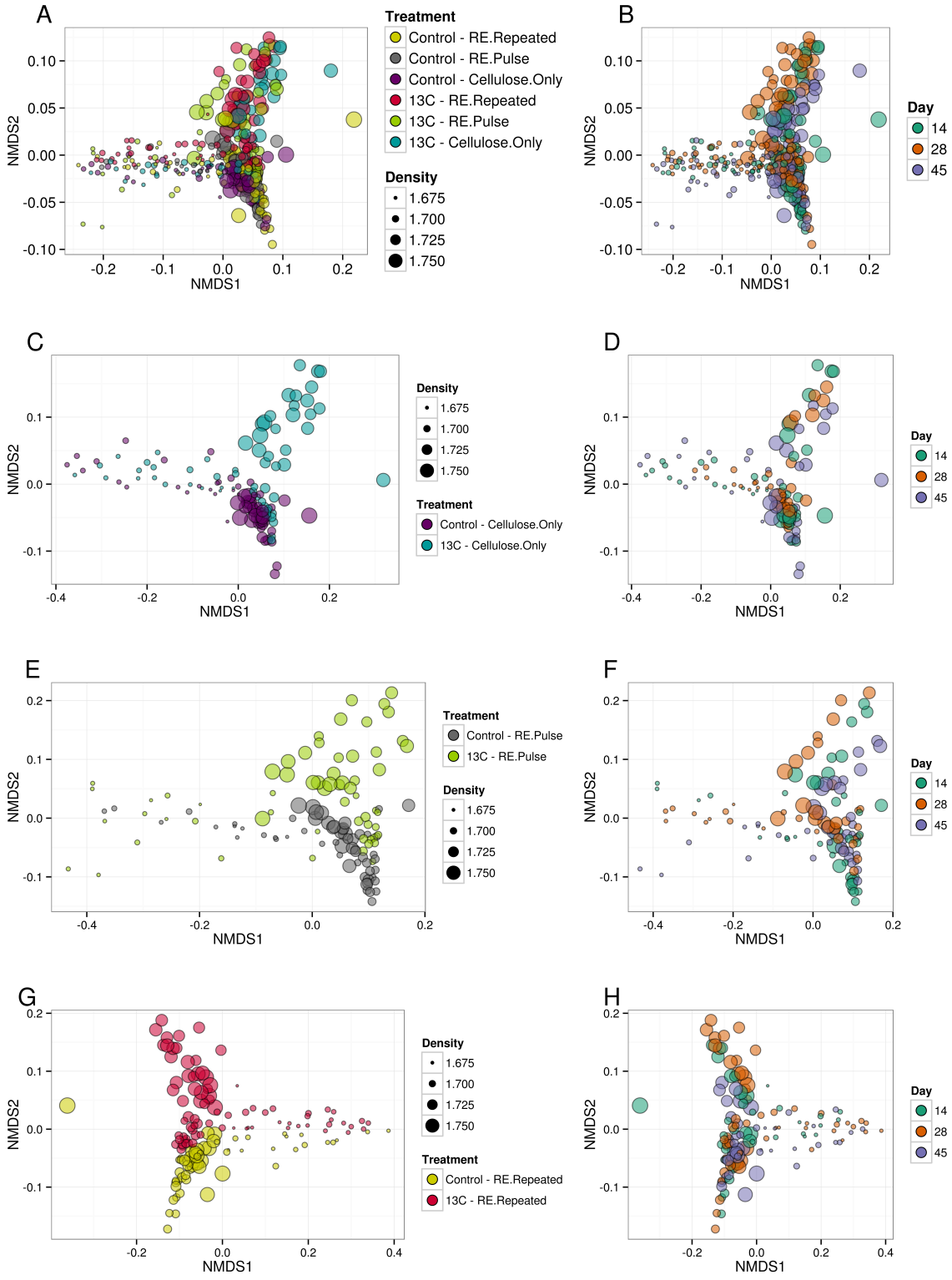
A
Treatment
Control - RE.Repeated
Control - RE.Pulse
Control - Cellulose.Only
13C - RE.Repeated
13C - RE.Pulse
13C - Cellulose.Only
Density
1.675
1.700
1.725
1.750
B
Day
14
28
45
C
D
E
F
G
H
NMDS1
NMDS2

Figure 4.5 NMDS analysis of 16S rRNA gene amplicon composition from density gradient fractions for: (A) all treatments, (C) the cellulose only treatment (control, purple; ^{13}C-cellulose, blue), (E) the root exudate pulse treatment (control, grey; ^{13}C-cellulose, green), (G) the repeated root exudate treatment (control, yellow; ^{13}C-cellulose, red), and each colored by day sample was harvested (B, D, F, and H). Fractions are sized according to their buoyant density. When members of the microbial community assimilate ^{13}C into their DNA their buoyant density increases. As a result, heavy density fractions (points with a 1.72 density or greater) from microcosms amended with ^{13}C-cellulose diverge from respective unlabeled-cellulose heavy density fractions and this result is significant for all treatments (Adonis, p-values: <0.001). The heavy density fractions within each ^{13}C-treatment have a significant time signature (Adonis, p-values: <0.05).

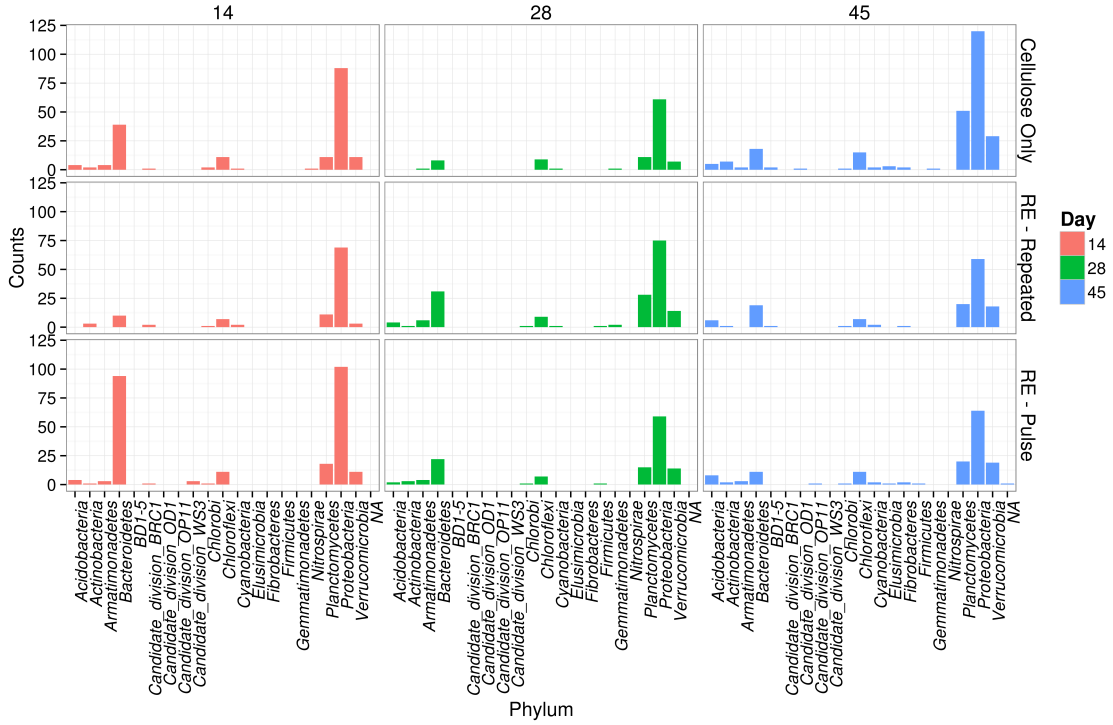

Figure 4.6 Counts of cellulose responders over time (days 14, 28, and 45) by phylum for the cellulose only treatment (top), repeated root exudate treatment (middle), and root exudate pulse treatment (bottom).

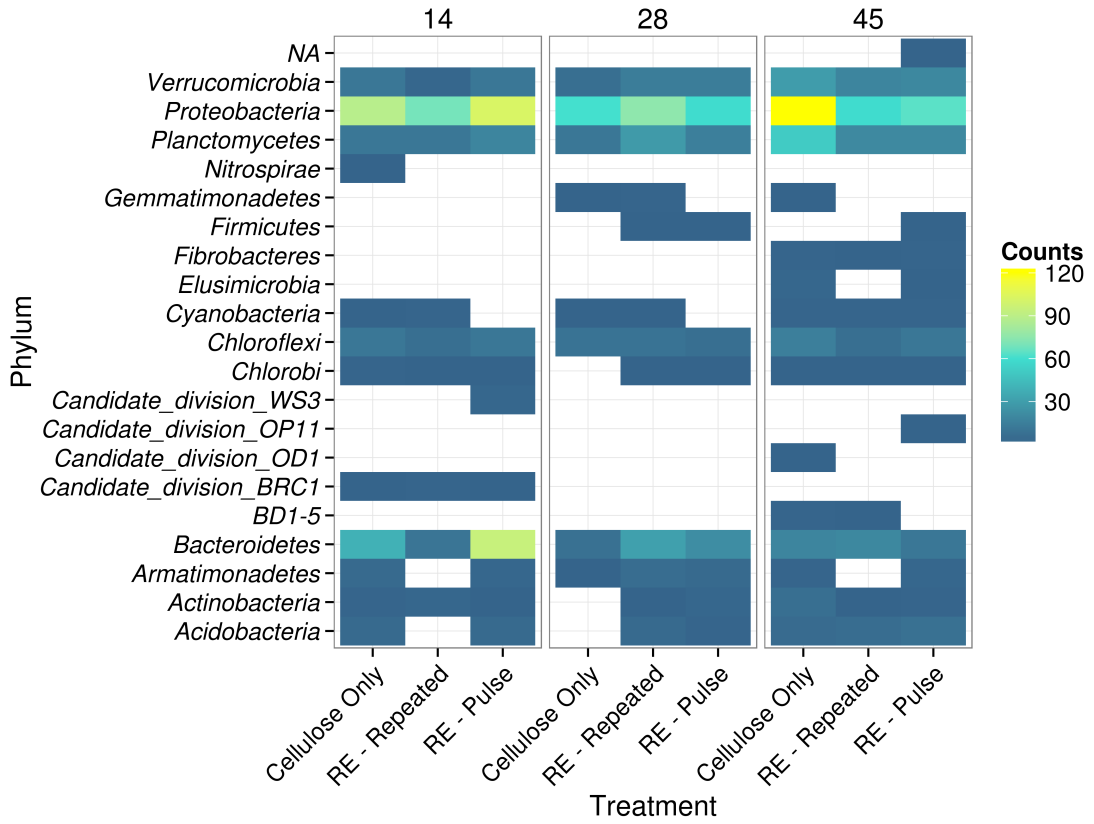

Figure 4.7 Counts of responders at days 14, 28, and 45 for each treatment.

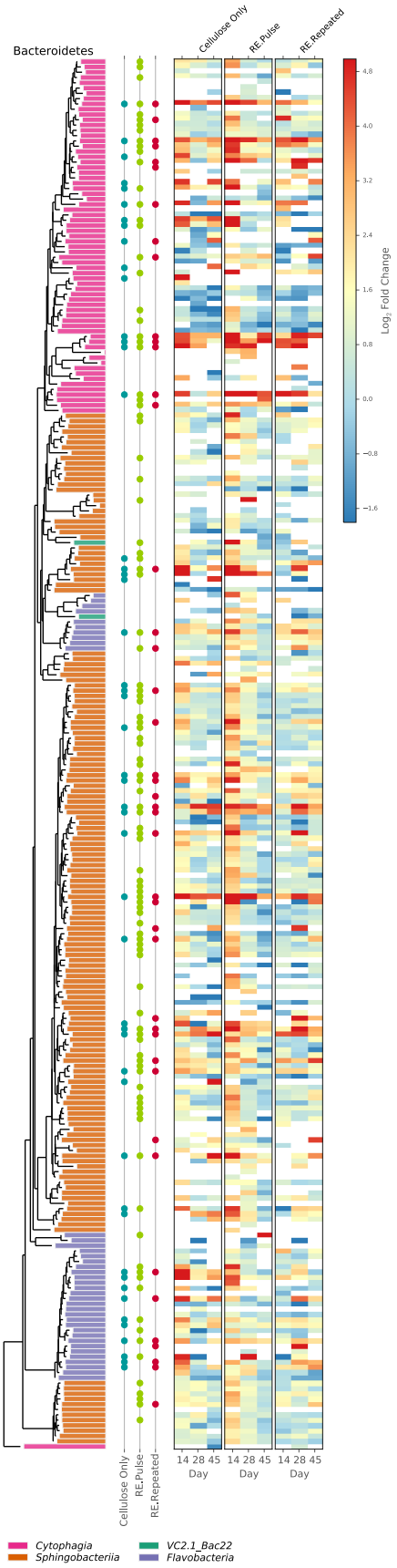

Figure 4.8 *Bacteroidetes* 16S rRNA gene phylogenetic tree colored by class. Points beside tips signify an OTU that significantly assimilated ^{13}C in the cellulose only treatment (blue), root exudate pulse treatment (green), or repeated root exudate treatment (red). Heat map indicates $\log_2$ fold change value for each OTU over time in the cellulose only treatment (left), root exudate pulse treatment (middle), and repeated root exudate treatment (right). Log_2 fold change represents the magnitude of enrichment in the ^{13}C-treatment relative to the control.

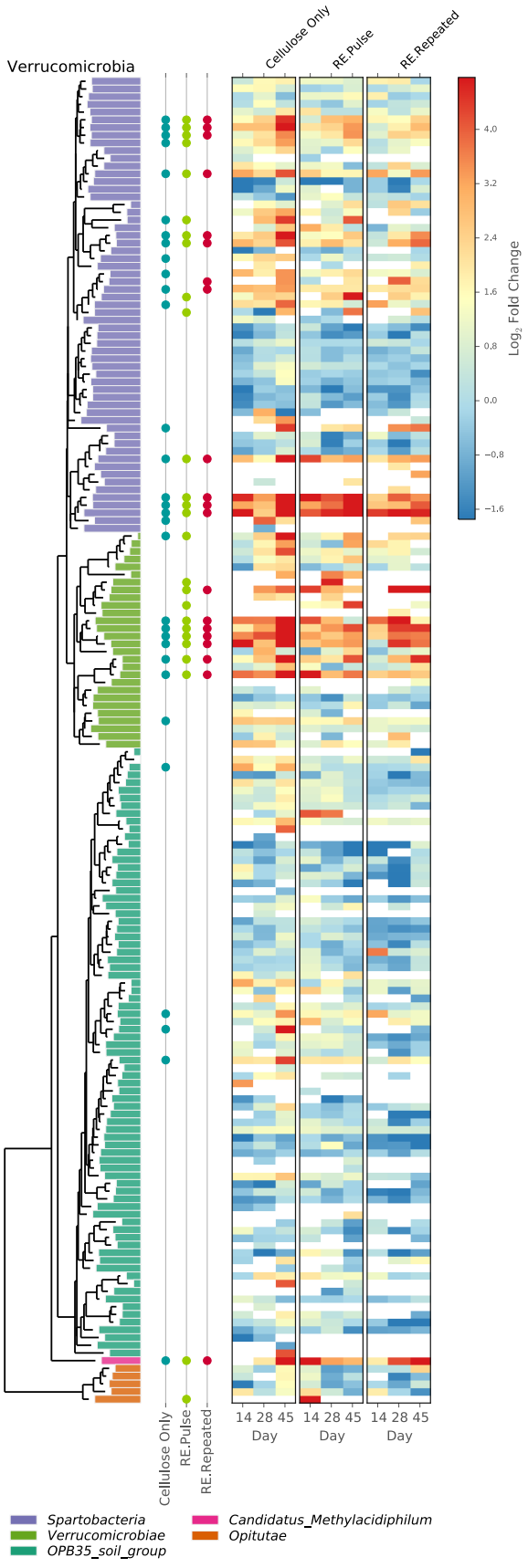

Figure 4.9 *Verrucomicrobia* 16S rRNA gene phylogenetic tree colored by class. Points beside tips signify an OTU that significantly assimilated ^{13}C in the cellulose only treatment (blue), root exudate pulse treatment (green), or repeated root exudate treatment (red). Heat map indicates log_2 fold change value for each OTU over time in the cellulose only treatment (left), root exudate pulse treatment (middle), and repeated root exudate treatment (right). Log_2 fold change represents the magnitude of enrichment in the ^{13}C-treatment relative to the control.

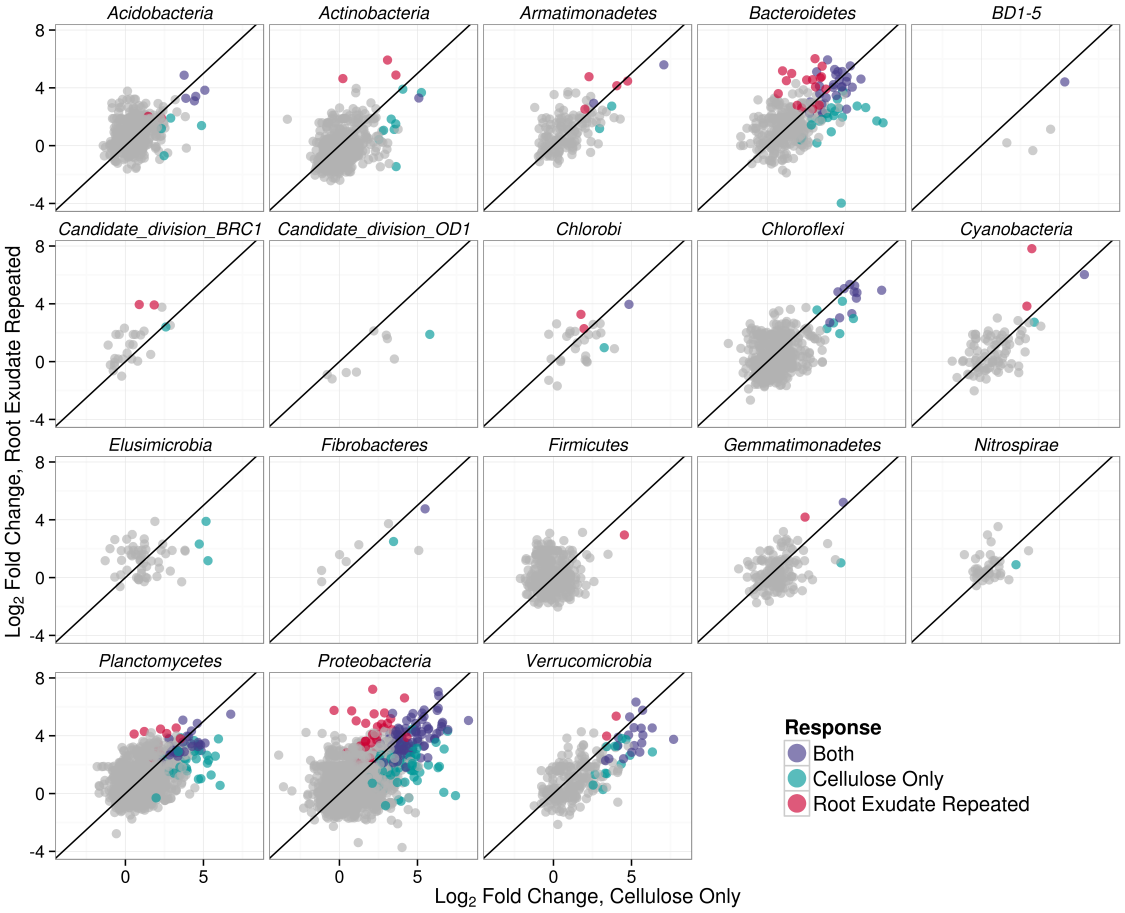

Figure 4.10 Linear regression of the maximum $\log_2$ fold change values for OTUs in the cellulose only treatment and the repeated root exudate treatment. Log_2 fold change value is the magnitude of enrichment for an OTU. OTUs are colored by the treatments it is considered a cellulose responder (none, grey; cellulose only, blue; root exudate repeated, red; both, purple). Black lines are a 1:1 reference line.

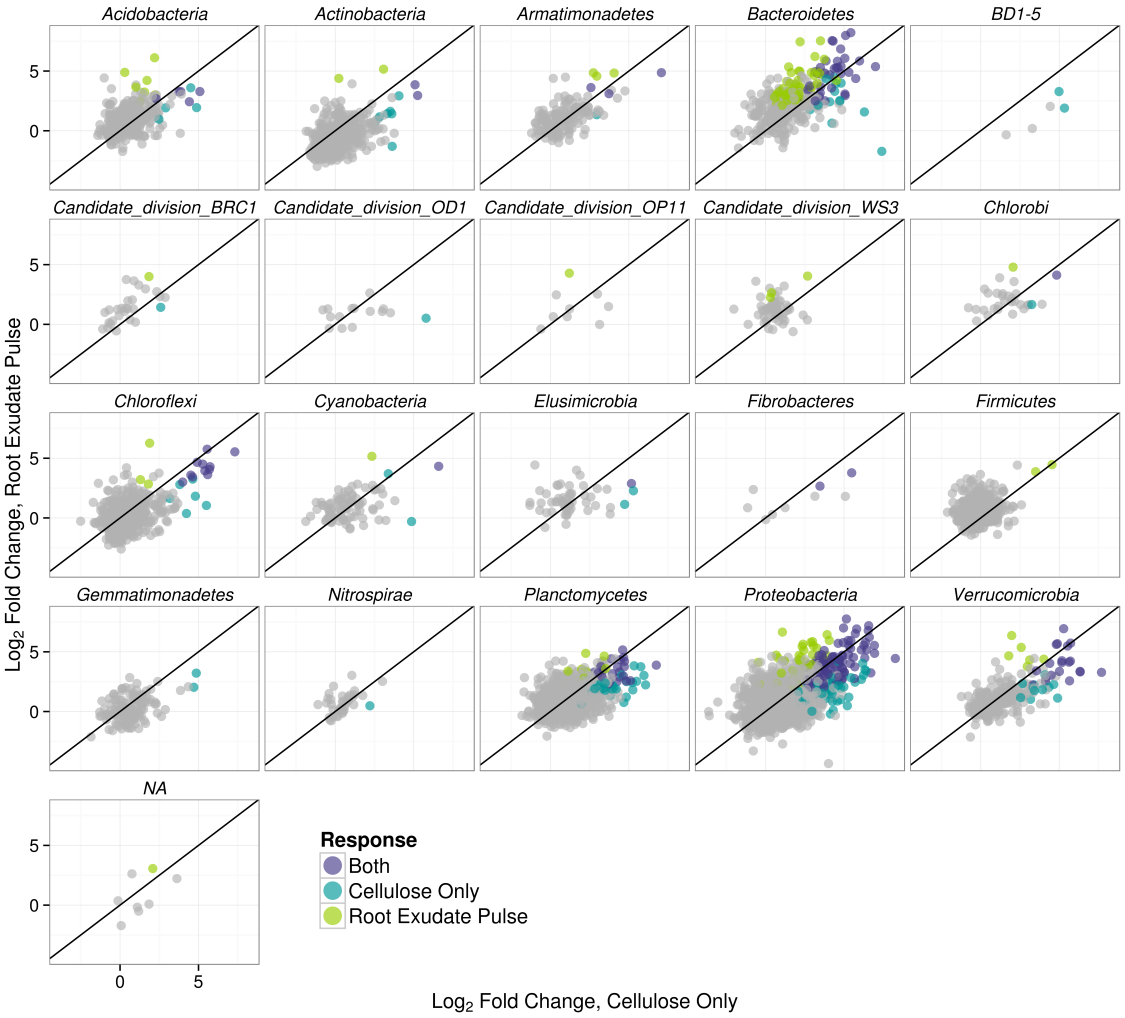

Figure 4.11 Linear regression of the maximum $\log_2$ fold change values for OTUs in the cellulose only treatment and the root exudate pulse treatment. Log_2 fold change value is the magnitude of enrichment for an OTU. OTUs are colored by the treatments it is considered a cellulose responder (none, grey; cellulose only, blue; root exudate pulse, green; both, purple). Black lines are a 1:1 reference line.

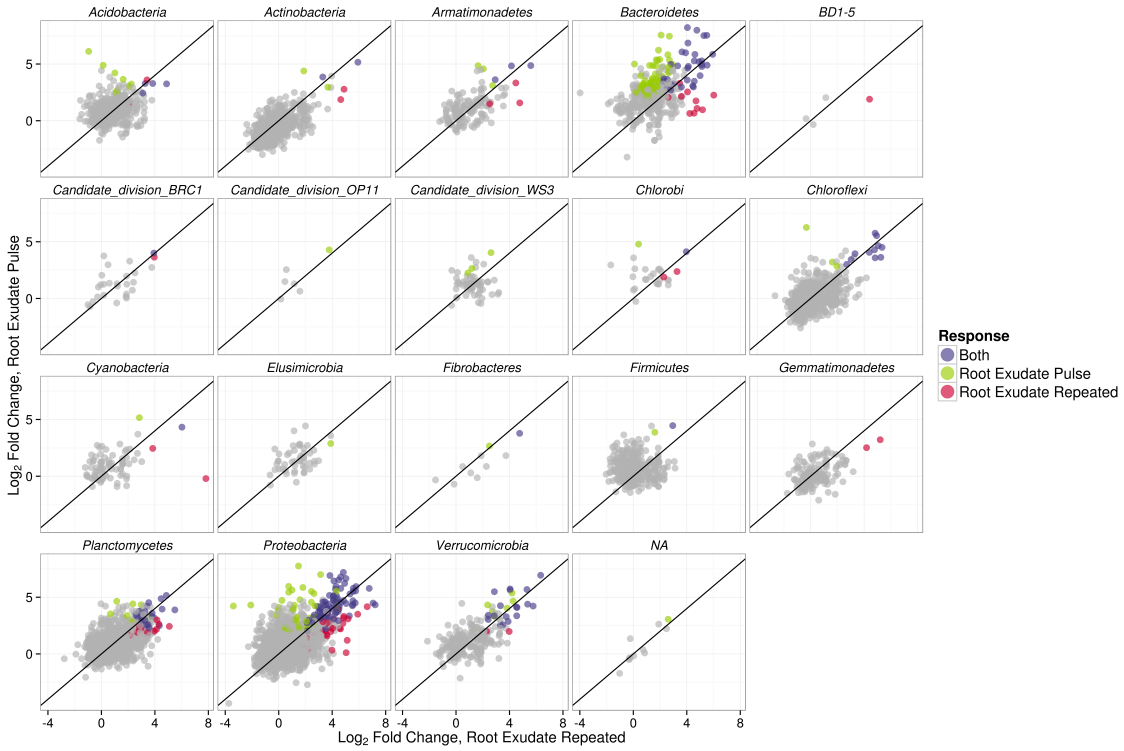

Figure 4.12 Linear regression of the maximum log_2 fold change values for OTUs in the root exudate pulse treatment and the repeated root exudate treatment. Log_2 fold change value is the magnitude of enrichment for an OTU. OTUs are colored by the treatments it is considered a cellulose responder (none, grey; root exudate pulse, green; root exudate repeated, red; both, purple). Black lines are a 1:1 reference line.

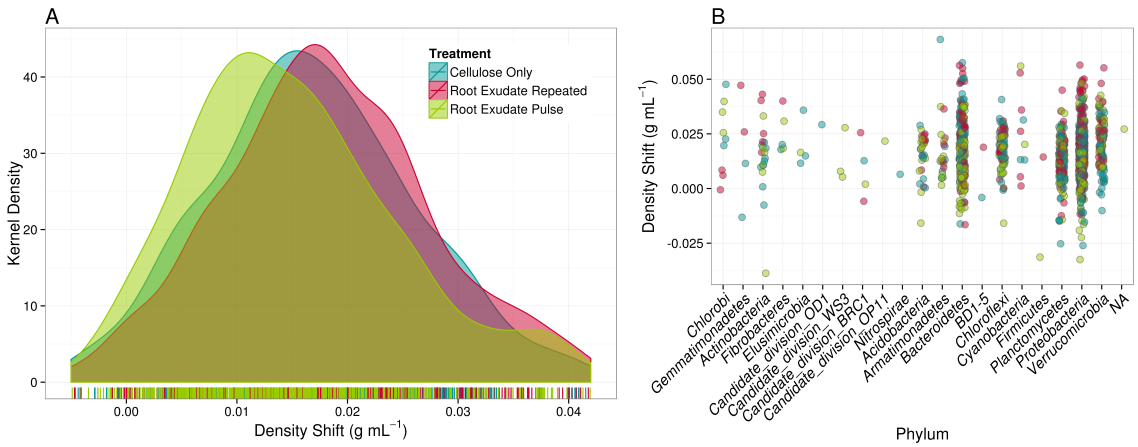

Figure 4.13 Density shifts of all responders for the cellulose only treatment (blue), repeated root exudate treatment (red), and root exudate pulse treatment (green)(A) and within each phylum (B). There were significant differences in density shifts of cellulose responders between all treatments (Wilcox, p-values: <0.001).

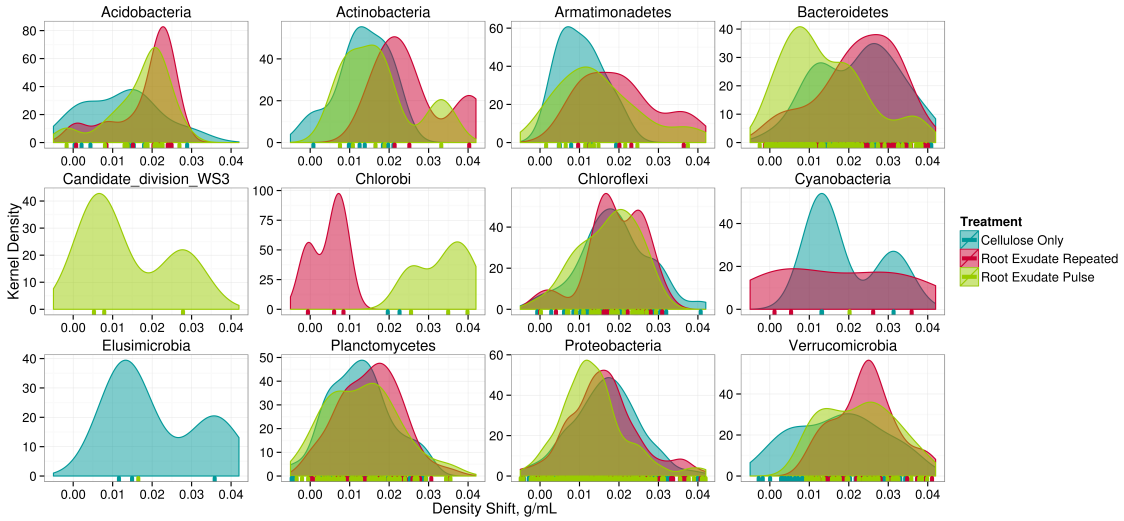

Figure 4.14 Density shifts within phyla of cellulose responders from cellulose only treatment (blue), repeated root exudate treatment (red), and root exudate pulse treatment (green).

4.9 Tables

Table 4.1 Soil carbon and nitrogen before and after incubations

Treatment	Total C	Total N	^{13}C-Cellulose	C:N
Soil (T_0)	11.88 ± 0.97	1.23 ± 0.10	-	9.61
Cellulose Only (T_f)	13.31 ± 0.88	1.45 ± 0.08	0.41 ± 0.15	9.20
Root Exudate Pulse (T_f)	12.52 ± 1.23	1.36 ± 0.13	0.24 ± 0.04	9.22
Root Exudate Repeated (T_f)	13.19 ± 0.81	1.43 ± 0.10	0.24 ± 0.05	9.23
Water Only (T_f)	12.82 ± 0.72	1.39 ± 0.06	-	9.23

Note: Values presented in this table represent both labeled and unlabeled series for each treatment except the ^{13}C-cellulose column, which represent values from only the ^{13}C-series for each treatment. Separate values (mean ± standard deviation) are presented in this table at the start (T_0) and end (T_f) of the experiment. All treatments started from the same soil (T_0), therefore, the T_0 value is representative of initial soil C and N for all treatments (before amendments). Total C for T_f values include any residual added C including cellulose. ^{13}C-cellulose column is how much of the C in the total C is cellulose derived. All treatments started with 0.89mg cellulose-^{13}C g^{-1} soil. All values are in units of mgC or mgN g^{-1} dry soil. The C:N values are calculated from a ratio of Total C:Total N.

Table 4.2: ^{13}C-cellulose responders in the cellulose only treatment

OTU ID	**Fold change** [a]	**Day** [b]	**Top BLAST hits** [c]	**BLAST %ID** [c]	**Phylum;Class;Order** [d]
OTU.10167	3.86	45	No hits of at least 97% identity	80.65	*Acidobacteria Candidatus-Chloracidobacterium uncultured-Acidobacteria-bacterium*
OTU.1236	5.07	45	No hits of at least 97% identity	82.02	*Acidobacteria Candidatus-Chloracidobacterium uncultured-bacterium*
OTU.7198	4.42	14	No hits of at least 97% identity	82.16	*Acidobacteria Candidatus-Chloracidobacterium uncultured-soil-bacterium*
OTU.3445	4.31	45	No hits of at least 97% identity	92.74	*Acidobacteria Candidatus-Solibacter uncultured-bacterium*
OTU.1707	2.89	45	No hits of at least 97% identity	86.97	*Acidobacteria Holophagae CA002*
OTU.7337	2.48	14	No hits of at least 97% identity	87.77	*Acidobacteria Holophagae iii1-8*
OTU.1576	4.87	14	No hits of at least 97% identity	90.59	*Acidobacteria Order-Incertae-Sedis Family-Incertae-Sedis*
OTU.1274	3.75	45	*Bryobacter aggregatus*	97.31	*Acidobacteria Order-Incertae-Sedis Family-Incertae-Sedis*
OTU.446	2.3	14	No hits of at least 97% identity	92.74	*Acidobacteria SJA-149 uncultured-bacterium*
OTU.1	2.53	14	*Arthrobacter spp.*	100.0	*Actinobacteria Micrococcales Micrococcaceae*
OTU.2360	3.62	45	*Micromonospora halophytica*, *Micromonospora lupini*, *Micromonospora chokoriensis*, *Micromonospora saelicesensis*, *Actinoplanes brasiliensis*, *Actinoplanes sp. A4029*, *Micromonospora carbonacea*	97.07	*Actinobacteria Micromonosporales Micromonosporaceae*
OTU.347	3.5	45	*Catellatospora coxensis*, *Catellatospora methionotrophica*, *Catellatospora chokoriensis*, *Catellatospora citrea subsp. citrea*	100.0	*Actinobacteria Micromonosporales Micromonosporaceae*
OTU.1598	2.82	45	*Aeromicrobium ponti*	98.66	*Actinobacteria Propionibacteriales Nocardioidaceae*
OTU.11083	4.05	14	*Saccharothrix sp. SA181*, *Saccharothrix longispora*, *Saccharothrix texasensis*	98.66	*Actinobacteria Pseudonocardiales Pseudonocardiaceae*
OTU.567	3.6	45	*Lentzea kentuckyensis*	100.0	*Actinobacteria Pseudonocardiales Pseudonocardiaceae*
OTU.101	3.31	45	*Streptomyces spp.*, *Kitasatospora spp.*	100.0	*Actinobacteria Streptomycetales Streptomycetaceae*
OTU.13276	5.23	45	No hits of at least 97% identity	90.91	*Actinobacteria Thermoleophilia Solirubrobacterales*
OTU.224	5.07	45	No hits of at least 97% identity	92.25	*Actinobacteria Thermoleophilia Solirubrobacterales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.720	3.73	14	No hits of at least 97% identity	94.67	*Armatimonadetes Armatimonadia Armatimonadales*
OTU.385	7.03	14	No hits of at least 97% identity	96.27	*Armatimonadetes uncultured-bacterium*
OTU.1219	2.95	14	No hits of at least 97% identity	85.83	*Armatimonadetes uncultured-bacterium*
OTU.519	2.58	14	No hits of at least 97% identity	91.8	*Armatimonadetes uncultured-bacterium*
OTU.1411	5.29	45	No hits of at least 97% identity	75.62	*BD1-5 uncultured-bacterium*
OTU.5258	4.94	45	No hits of at least 97% identity	75.27	*BD1-5 uncultured-bacterium*
OTU.1647	7.38	14	*Hymenobacter ocellatus*	97.3	*Bacteroidetes Cytophagia Cytophagales*
OTU.879	5.42	14	No hits of at least 97% identity	94.09	*Bacteroidetes Cytophagia Cytophagales*
OTU.1269	5.29	14	*Dyadobacter hamtensis*	98.38	*Bacteroidetes Cytophagia Cytophagales*
OTU.802	4.77	14	No hits of at least 97% identity	90.59	*Bacteroidetes Cytophagia Cytophagales*
OTU.10789	4.66	14	No hits of at least 97% identity	95.95	*Bacteroidetes Cytophagia Cytophagales*
OTU.10228	4.57	14	No hits of at least 97% identity	91.39	*Bacteroidetes Cytophagia Cytophagales*
OTU.1512	4.51	14	No hits of at least 97% identity	90.19	*Bacteroidetes Cytophagia Cytophagales*
OTU.2730	4.47	14	No hits of at least 97% identity	95.42	*Bacteroidetes Cytophagia Cytophagales*
OTU.1120	4.41	14	No hits of at least 97% identity	96.76	*Bacteroidetes Cytophagia Cytophagales*
OTU.259	4.37	14	*Dyadobacter beijingensis*	97.57	*Bacteroidetes Cytophagia Cytophagales*
OTU.389	4.29	14	No hits of at least 97% identity	94.35	*Bacteroidetes Cytophagia Cytophagales*
OTU.500	4.23	14	No hits of at least 97% identity	90.59	*Bacteroidetes Cytophagia Cytophagales*
OTU.1498	4.21	45	No hits of at least 97% identity	92.1	*Bacteroidetes Cytophagia Cytophagales*
OTU.1847	4.06	45	No hits of at least 97% identity	85.71	*Bacteroidetes Cytophagia Cytophagales*
OTU.814	3.49	14	No hits of at least 97% identity	95.42	*Bacteroidetes Cytophagia Cytophagales*
OTU.1419	5.74	14	*Flavobacterium glycines*	98.37	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.336	5.07	14	*Flavobacterium banpakuense*	99.46	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.1448	4.8	14	*Flavobacterium beibuense*	98.38	*Bacteroidetes Flavobacteria Flavobacteriales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.783	4.73	14	*Flavobacterium johnsoniae*	98.35	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.3141	4.13	14	*Flavobacterium chungnamense*	97.55	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.3519	3.78	14	*Flavobacterium spp.*	97.28	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.237	3.48	14	*Flavobacterium columnare*	98.08	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.6303	3.34	14	No hits of at least 97% identity	96.47	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.580	3.32	45	No hits of at least 97% identity	87.06	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.7456	3.16	14	*Flavobacterium granuli*	98.88	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.1171	2.26	14	*Flavobacterium granuli*, *Flavobacterium frigidimaris*	97.57	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.2004	6.98	14	*Pedobacter borealis*, *Pedobacter agri PB92*	100.0	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1862	6.25	45	No hits of at least 97% identity	93.51	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1470	5.97	14	*Mucilaginibacter sp. DRP28*, *Mucilaginibacter gossypiicola*, *Mucilaginibacter gossypii*	100.0	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.142	5.09	14	No hits of at least 97% identity	96.22	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.577	5.04	45	*Chitinophaga japonensis*	98.38	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1032	5.03	45	No hits of at least 97% identity	92.37	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.102	4.75	28	*Niastella koreensis*	99.19	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.2157	4.71	45	*Sphingobacterium detergens*	100.0	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.12560	4.31	14	*Chitinophaga niabensis*	98.64	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.5424	4.2	45	*Niastella populi*	97.84	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.659	4.08	14	No hits of at least 97% identity	87.98	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.877	3.85	45	No hits of at least 97% identity	88.92	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.534	3.78	28	*Chitinophaga filiformis*, *Chitinophaga ginsengisoli*	98.38	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7770	3.76	14	*Niabella yanshanensis*	97.3	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.4278	3.52	14	*Terrimonas lutea*	97.57	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.170	3.42	14	No hits of at least 97% identity	95.41	*Bacteroidetes Sphingobacteriia Sphingobacteriales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1916	3.31	14	No hits of at least 97% identity	95.68	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1009	3.15	14	*Lacibacter cauensis*	98.38	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7805	2.81	14	*Terrimonas lutea*	97.77	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.12647	2.73	14	*Flavisolibacter ginsengisoli*	97.28	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.428	2.69	14	*Terrimonas ferruginea*	97.84	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.4	2.2	14	No hits of at least 97% identity	95.95	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.378	2.13	14	*Pedobacter oryzae DSM 19973*	98.65	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.694	2.59	14	No hits of at least 97% identity	82.23	*Candidate-division-BRC1 uncultured-bacterium*
OTU.2028	5.74	45	No hits of at least 97% identity	80.33	*Candidate-division-OD1 uncultured-bacterium*
OTU.1342	4.84	14	No hits of at least 97% identity	83.52	*Chlorobi Chlorobia Chlorobiales*
OTU.941	3.25	14	No hits of at least 97% identity	82.45	*Chlorobi Chlorobia Chlorobiales*
OTU.1546	5.6	28	No hits of at least 97% identity	80.95	*Chloroflexi Anaerolineae Anaerolineales*
OTU.2440	5.45	45	No hits of at least 97% identity	80.9	*Chloroflexi Anaerolineae Anaerolineales*
OTU.1506	5.39	45	No hits of at least 97% identity	91.06	*Chloroflexi Anaerolineae Anaerolineales*
OTU.8564	4.76	45	No hits of at least 97% identity	80.5	*Chloroflexi Anaerolineae Anaerolineales*
OTU.1551	4.62	45	No hits of at least 97% identity	80.11	*Chloroflexi Anaerolineae Anaerolineales*
OTU.553	4.62	14	No hits of at least 97% identity	84.54	*Chloroflexi Anaerolineae Anaerolineales*
OTU.468	4.51	14	No hits of at least 97% identity	79.95	*Chloroflexi Anaerolineae Anaerolineales*
OTU.3038	4.2	45	No hits of at least 97% identity	81.7	*Chloroflexi Anaerolineae Anaerolineales*
OTU.535	3.98	45	No hits of at least 97% identity	75.07	*Chloroflexi Anaerolineae Anaerolineales*
OTU.2050	3.78	45	No hits of at least 97% identity	89.23	*Chloroflexi Anaerolineae Anaerolineales*
OTU.1703	3.17	14	No hits of at least 97% identity	80.32	*Chloroflexi Anaerolineae Anaerolineales*
OTU.1307	6.98	45	No hits of at least 97% identity	88.65	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.245	5.68	45	No hits of at least 97% identity	89.68	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.5834	5.54	45	No hits of at least 97% identity	88.62	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.6853	5.26	45	No hits of at least 97% identity	88.89	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.6205	4.9	45	No hits of at least 97% identity	88.42	*Chloroflexi Herpetosiphonales Herpetosiphonaceae*
OTU.50	5.58	14	No hits of at least 97% identity	84.91	*Chloroflexi Ktedonobacteria C0119*
OTU.1629	3.36	45	No hits of at least 97% identity	86.33	*Cyanobacteria MLE1-12 uncultured-bacterium*
OTU.1155	6.39	28	No hits of at least 97% identity	94.86	*Cyanobacteria SM1D11 uncultured-bacterium*
OTU.5011	4.84	14	No hits of at least 97% identity	93.78	*Cyanobacteria SM1D11 uncultured-bacterium*
OTU.3573	5.22	45	No hits of at least 97% identity	83.29	*Elusimicrobia Lineage-IV uncultured-bacterium*
OTU.2537	5.12	45	No hits of at least 97% identity	83.02	*Elusimicrobia Lineage-IV*
OTU.6775	4.7	45	No hits of at least 97% identity	84.89	*Elusimicrobia MVP-88*
OTU.3241	5.46	45	No hits of at least 97% identity	80.55	*Fibrobacteres Fibrobacteria Fibrobacterales*
OTU.1639	3.46	45	No hits of at least 97% identity	81.27	*Fibrobacteres Fibrobacteria Fibrobacterales*
OTU.1102	4.69	45	No hits of at least 97% identity	85.19	*Gemmatimonadetes AT425-EubC11-terrestrial-group uncultured-bacterium*
OTU.2772	4.69	28	No hits of at least 97% identity	92.27	*Gemmatimonadetes Gemmatimonadales Gemmatimonadaceae*
OTU.763	2.21	14	No hits of at least 97% identity	84.59	*Nitrospirae Nitrospira Nitrospirales*
OTU.4075	4.65	14	No hits of at least 97% identity	83.38	*Planctomycetes OM190 uncultured-bacterium*
OTU.6795	4.59	14	No hits of at least 97% identity	83.52	*Planctomycetes OM190 uncultured-bacterium*
OTU.549	3.92	28	No hits of at least 97% identity	81.32	*Planctomycetes OM190 uncultured-bacterium*
OTU.8807	3.47	45	No hits of at least 97% identity	81.87	*Planctomycetes OM190 uncultured-bacterium*
OTU.1029	3.42	28	No hits of at least 97% identity	82.57	*Planctomycetes OM190 uncultured-bacterium*
OTU.312	2.79	14	No hits of at least 97% identity	82.88	*Planctomycetes OM190 uncultured-bacterium*
OTU.3216	4.01	28	No hits of at least 97% identity	81.75	*Planctomycetes OM190*
OTU.595	2.91	28	No hits of at least 97% identity	83.33	*Planctomycetes OM190*
OTU.494	2.4	14	No hits of at least 97% identity	81.62	*Planctomycetes OM190*
OTU.3073	5.15	45	No hits of at least 97% identity	79.58	*Planctomycetes Phycisphaerae Phycisphaerales*
OTU.1068	4.22	45	No hits of at least 97% identity	77.89	*Planctomycetes Phycisphaerae Phycisphaerales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1152	3.93	45	No hits of at least 97% identity	83.03	*Planctomycetes Phycisphaerae Phycisphaerales*
OTU.1077	3.81	45	No hits of at least 97% identity	84.39	*Planctomycetes Phycisphaerae Phycisphaerales*
OTU.1334	3.11	45	No hits of at least 97% identity	77.93	*Planctomycetes Phycisphaerae Phycisphaerales*
OTU.678	4.8	45	No hits of at least 97% identity	78.59	*Planctomycetes Phycisphaerae WD2101-soil-group*
OTU.3000	4.5	45	No hits of at least 97% identity	79.17	*Planctomycetes Phycisphaerae WD2101-soil-group*
OTU.1324	3.5	45	No hits of at least 97% identity	80.82	*Planctomycetes Phycisphaerae WD2101-soil-group*
OTU.307	2.62	28	No hits of at least 97% identity	80.06	*Planctomycetes Phycisphaerae WD2101-soil-group*
OTU.266	6.73	45	No hits of at least 97% identity	85.07	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2901	5.98	45	No hits of at least 97% identity	92.53	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.3667	5.88	45	No hits of at least 97% identity	83.99	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.9627	5.7	45	*Schlesneria paludicola*	97.04	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1702	5.46	45	No hits of at least 97% identity	86.67	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2880	5.3	45	No hits of at least 97% identity	86.98	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1650	4.84	45	No hits of at least 97% identity	88.8	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.3091	4.81	45	No hits of at least 97% identity	89.63	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1294	4.7	45	No hits of at least 97% identity	91.44	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.701	4.64	45	No hits of at least 97% identity	86.46	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2441	4.64	45	No hits of at least 97% identity	89.36	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.10699	4.44	14	No hits of at least 97% identity	84.17	*Planctomycetes Planctomycetacia Planctomycetales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1816	4.37	45	No hits of at least 97% identity	91.71	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.667	4.27	45	No hits of at least 97% identity	89.3	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1869	4.26	45	No hits of at least 97% identity	86.13	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.521	4.18	45	No hits of at least 97% identity	87.97	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.559	3.91	45	No hits of at least 97% identity	86.74	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2586	3.86	45	No hits of at least 97% identity	85.08	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.3835	3.81	14	No hits of at least 97% identity	86.86	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.600	3.77	45	No hits of at least 97% identity	93.57	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1370	3.67	45	No hits of at least 97% identity	87.85	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.633	3.67	45	No hits of at least 97% identity	92.25	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1174	3.52	45	No hits of at least 97% identity	92.03	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.4923	3.48	45	No hits of at least 97% identity	88.06	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2151	3.38	28	No hits of at least 97% identity	88.53	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.80	3.35	45	No hits of at least 97% identity	90.93	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.8906	3.35	28	No hits of at least 97% identity	89.92	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2102	3.34	45	No hits of at least 97% identity	89.04	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.404	3.29	45	No hits of at least 97% identity	90.05	*Planctomycetes Planctomycetacia Planctomycetales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.438	3.21	45	No hits of at least 97% identity	92.25	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1338	3.14	45	No hits of at least 97% identity	90.08	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.445	3.09	45	No hits of at least 97% identity	89.25	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1239	3.04	45	No hits of at least 97% identity	90.43	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.513	3.03	45	No hits of at least 97% identity	95.16	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.6443	3.03	45	No hits of at least 97% identity	92.09	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.202	2.84	45	No hits of at least 97% identity	86.74	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.8826	2.82	45	No hits of at least 97% identity	86.1	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.685	2.77	28	No hits of at least 97% identity	87.8	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.368	2.74	45	No hits of at least 97% identity	89.52	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.238	2.74	45	No hits of at least 97% identity	88.03	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.7136	2.3	28	No hits of at least 97% identity	90.3	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.825	1.97	14	No hits of at least 97% identity	93.63	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.213	1.85	28	No hits of at least 97% identity	91.44	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.623	5.09	45	No hits of at least 97% identity	85.56	*Planctomycetes vadinHA49 uncultured-Planctomycetales-bacterium*
OTU.1329	5.23	45	No hits of at least 97% identity	81.47	*Planctomycetes vadinHA49 uncultured-bacterium*
OTU.2136	4.68	45	No hits of at least 97% identity	85.05	*Planctomycetes vadinHA49 uncultured-bacterium*
OTU.690	6.93	14	*Brevundimonas variabilis*	99.46	*Proteobacteria Alphaproteobacteria Caulobacterales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.2416	6.22	14	*Brevundimonas vesicularis*, *Brevundimonas nasdae*	100.0	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.3062	6.09	14	*Caulobacter vibrioides*, *Caulobacter segnis*	99.46	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.184	5.8	14	*Brevundimonas alba*	99.19	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.10	5.26	14	*Caulobacter henricii*	99.46	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.232	4.88	14	*Asticcacaulis taihuensis*	98.13	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.3030	4.86	28	*Asticcacaulis excentricus*	98.66	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.402	4.26	14	*Caulobacter fusiformis*	99.46	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.12989	3.94	14	*Brevundimonas terrae*	99.46	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.121	3.33	14	*Phenylobacterium sp. A8*	98.66	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.566	3.29	14	*Phenylobacterium lituiforme*	97.31	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.12754	3.09	14	*Phenylobacterium sp. A8*	98.61	*Proteobacteria Alphaproteobacteria Caulobacterales*
OTU.3134	5.24	45	No hits of at least 97% identity	85.03	*Proteobacteria Alphaproteobacteria DB1-14*
OTU.371	4.83	45	No hits of at least 97% identity	88.27	*Proteobacteria Alphaproteobacteria DB1-14*
OTU.1248	4.87	14	*Ferrovibrio denitrificans*	98.92	*Proteobacteria Alphaproteobacteria MNG3*
OTU.277	6.31	45	No hits of at least 97% identity	94.09	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.34	4.68	45	*Ensifer adhaerens*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.111	4.44	45	No hits of at least 97% identity	92.23	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.472	4.39	45	No hits of at least 97% identity	92.23	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.3911	4.38	45	*Rhizobium spp.*, *Arthrobacter spp.*	97.83	*Proteobacteria Alphaproteobacteria Rhizobiales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.7	4.25	45	*Bradyrhizobium spp.*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.5106	4.24	45	*Afipia massiliensis*	98.12	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.73	4.12	14	*Bosea sp. R-46060*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.10638	4.11	14	*Aminobacter sp. STM 4645*	98.37	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.16	4.11	45	*Mesorhizobium caraganae*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.9449	3.98	14	No hits of at least 97% identity	96.47	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.49	3.95	45	*Rhizobium herbae*	99.73	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.27	3.82	28	*Devosia insulae*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.5674	3.77	45	*Bosea sp. R-46070*	99.73	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.844	3.76	45	*Devosia limi*	98.12	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.86	3.72	45	No hits of at least 97% identity	94.62	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.6165	3.69	45	*Rhizobium cellulosilyticum*	98.92	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1098	3.49	14	*Vasilyevaea enhydra*	98.66	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1759	3.28	45	*Shinella yambaruensis*	99.18	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.264	2.8	45	*Aminobacter aminovorans*, *Mesorhizobium loti*, *Mesorhizobium australicum WSM2073*, *Mesorhizobium shangrilense*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.3368	2.72	45	*Rhodopseudomonas sp. R-45977*	98.66	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.512	2.65	45	No hits of at least 97% identity	94.64	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.6819	2.56	14	No hits of at least 97% identity	96.74	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.11661	2.49	14	No hits of at least 97% identity	94.59	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.107	2.18	45	*Rhodoplanes roseus*	97.31	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.6217	1.86	14	No hits of at least 97% identity	95.65	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1057	3.53	14	*Catellibacterium nectariphilum*	98.39	*Proteobacteria Alphaproteobacteria Rhodobacterales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.338	7.96	45	No hits of at least 97% identity	89.84	*Proteobacteria Alphaproteobacteria Rhodospirillales*
OTU.2418	6.66	14	No hits of at least 97% identity	86.74	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.766	4.65	14	No hits of at least 97% identity	91.91	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.1480	4.1	14	No hits of at least 97% identity	91.67	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.3165	6.61	14	No hits of at least 97% identity	96.78	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.31	6.56	14	*Sphingomonas trueperi*, *Sphingomonas pituitosa*	98.66	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.169	5.7	45	*Sphingopyxis panaciterrae*, *Sphingopyxis chilensis*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.7891	5.38	45	*Sphingomonas haloaromaticamans*, *Sphingomonas wittichii*	97.86	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.4687	5.37	45	*Sphingomonas asaccharolytica*	97.86	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.380	5.3	14	No hits of at least 97% identity	95.98	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.9193	4.95	14	No hits of at least 97% identity	96.51	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.10665	4.93	14	No hits of at least 97% identity	94.72	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.8290	4.66	14	*Sphingomonas koreensis*	97.59	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.2256	4.61	45	*Sphingomonas sp. 382*, *Sphingomonas wittichii*, *Sphingomonas sp. UM2*	98.39	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.456	3.69	45	*Novosphingobium nitrogenifigens*	98.39	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.426	3.48	28	*Altererythrobacter sp. S3-63*	97.86	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.8359	3.45	45	*Kaistobacter terrae*	98.12	*Proteobacteria Alphaproteobacteria Sphingomonadales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.8167	2.99	14	No hits of at least 97% identity	92.8	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.4714	2.94	45	*Erythrobacter aquimaris*	98.39	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.740	2.75	28	*Novosphingobium lentum*	98.93	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.23	2.67	28	*Sphingomonas sp. YC6722*	97.32	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.420	2.66	28	*Kaistobacter terrae*	98.12	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.6783	2.52	28	No hits of at least 97% identity	96.94	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.13	2.36	28	*Sphingomonas jaspsi*	98.93	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.9664	2.06	28	No hits of at least 97% identity	96.27	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.3156	2.03	28	*Kaistobacter terrae*	97.32	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.5320	1.72	14	*Kaistobacter terrae*	97.06	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.151	6.44	14	*Polaromonas aquatica*, *Polaromonas jejuensis*, *Polaromonas vacuolata*	99.46	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.4112	5.56	45	*Ralstonia insidiosa*	97.06	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.1666	5.19	45	*Cupriavidus necator*, *Wautersia numazuensis*, *Cupriavidus basilensis*	99.73	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5544	4.93	45	*Herminiimonas aquatilis*	97.58	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5575	4.91	14	*Paucibacter toxinivorans*	98.66	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.72	4.87	14	*Rhizobacter dauci*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.54	4.27	14	*Pseudoduganella violaceinigra*	99.73	*Proteobacteria Betaproteobacteria Burkholderiales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.815	4.16	45	No hits of at least 97% identity	94.89	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.11076	4.11	14	*Variovorax ginsengisoli*, *Variovorax boronicumulans*	97.3	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5105	3.87	14	*Variovorax paradoxus*	98.12	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.159	3.81	14	*Massilia suwonensis*, *Massilia alkalitolerans*, *Massilia jejuensis*, *Massilia varians*, *Massilia niabensis*	100.0	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.7831	3.28	14	*Massilia tieshanensis*, *Massilia aerilata*	98.92	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.139	3.21	45	*Pelomonas puraquae*	98.66	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.441	3.17	45	No hits of at least 97% identity	93.82	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.2645	2.78	45	*Herbaspirillum sp. SUEMI08*	97.85	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.40	2.56	14	*Acidovorax sp. NF1078*	99.73	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5914	2.35	14	*Comamonas thiooxydans*, *Comamonas testosteroni*	98.39	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.2176	1.71	14	*Piscinibacter aquaticus*	98.91	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.2161	4.73	45	No hits of at least 97% identity	90.11	*Proteobacteria Betaproteobacteria Hydrogenophilales*
OTU.357	5.59	45	No hits of at least 97% identity	94.93	*Proteobacteria Betaproteobacteria Nitrosomonadales*
OTU.965	3.58	45	No hits of at least 97% identity	93.03	*Proteobacteria Betaproteobacteria Nitrosomonadales*
OTU.1967	6.29	14	No hits of at least 97% identity	91.18	*Proteobacteria Betaproteobacteria Rhodocyclales*
OTU.2276	6.33	45	No hits of at least 97% identity	91.71	*Proteobacteria Deltaproteobacteria Bdellovibrionales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.6149	5.66	45	No hits of at least 97% identity	93.32	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.913	5.43	45	No hits of at least 97% identity	94.12	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.4322	4.62	45	No hits of at least 97% identity	89.57	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.699	3.73	14	No hits of at least 97% identity	93.32	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.2525	3.43	45	No hits of at least 97% identity	86.17	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.4156	4.63	45	No hits of at least 97% identity	86.03	*Proteobacteria Deltaproteobacteria GR-WP33-30*
OTU.686	6.89	45	No hits of at least 97% identity	92.27	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1041	6.09	45	No hits of at least 97% identity	84.0	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.3831	5.86	45	No hits of at least 97% identity	90.91	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.706	5.65	45	No hits of at least 97% identity	96.52	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.649	5.27	45	No hits of at least 97% identity	92.51	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.461	5.01	45	No hits of at least 97% identity	91.71	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.6791	5.01	45	No hits of at least 97% identity	91.33	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.947	4.89	45	No hits of at least 97% identity	90.67	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.2624	4.88	45	No hits of at least 97% identity	89.07	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.269	4.88	45	No hits of at least 97% identity	89.25	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.883	4.65	45	No hits of at least 97% identity	90.93	*Proteobacteria Deltaproteobacteria Myxococcales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.2610	4.58	45	No hits of at least 97% identity	83.96	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1097	4.56	45	*Cystobacter violaceus*, *Archangium gephyra*	97.59	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.2576	4.28	45	No hits of at least 97% identity	85.94	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1889	4.22	45	No hits of at least 97% identity	95.21	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.5612	4.15	45	No hits of at least 97% identity	95.99	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.2628	4.11	45	No hits of at least 97% identity	90.11	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.10767	4.08	45	No hits of at least 97% identity	91.06	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.2179	3.98	45	No hits of at least 97% identity	90.16	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.3805	3.76	45	No hits of at least 97% identity	89.7	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.722	3.67	45	No hits of at least 97% identity	90.64	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1900	3.65	45	No hits of at least 97% identity	92.51	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.2493	3.52	45	No hits of at least 97% identity	84.95	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.6077	3.5	28	No hits of at least 97% identity	92.78	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1573	3.48	45	No hits of at least 97% identity	89.28	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.3842	3.3	45	No hits of at least 97% identity	90.4	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.1736	3.28	45	No hits of at least 97% identity	82.62	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.927	2.95	45	*Enhygromyxa salina*	97.05	*Proteobacteria Deltaproteobacteria Myxococcales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1398	4.56	45	No hits of at least 97% identity	87.5	*Proteobacteria Deltaproteobacteria Sh765B-TzT-29*
OTU.2212	7.35	45	No hits of at least 97% identity	90.11	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.537	6.34	14	No hits of at least 97% identity	93.01	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.1223	6.25	45	No hits of at least 97% identity	96.79	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.2386	4.74	45	No hits of at least 97% identity	87.23	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.4746	4.66	45	No hits of at least 97% identity	92.51	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.2329	4.62	45	No hits of at least 97% identity	93.85	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.1318	4.55	45	No hits of at least 97% identity	91.98	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.584	4.49	45	No hits of at least 97% identity	89.3	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.2213	4.43	45	No hits of at least 97% identity	95.99	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.3661	3.96	45	No hits of at least 97% identity	95.99	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.3267	3.78	14	No hits of at least 97% identity	95.43	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.811	3.59	45	No hits of at least 97% identity	93.85	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.1083	3.18	45	*Legionella sp. LegA*	97.86	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.1648	4.55	45	No hits of at least 97% identity	87.98	*Proteobacteria Gammaproteobacteria NKB5*
OTU.131	6.7	14	*Cellvibrio ostraviensis*, *Cellvibrio fibrivorans*, *Cellvibrio mixtus subsp. mixtus*	100.0	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.1590	6.6	14	*Cellvibrio gandavensis*	97.59	*Proteobacteria Gammaproteobacteria Pseudomonadales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.11032	5.14	14	*Cellvibrio gandavensis*	99.46	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.3310	4.36	45	No hits of at least 97% identity	92.25	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.6689	3.83	45	*Sphingomonas sp. S8-3*	99.46	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.7738	3.71	14	*Pseudomonas brassicacearum subsp. neoaurantiaca*, *Pseudomonas frederiksbergensis*	98.64,	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.1214	3.22	14	*Pseudomonas alcaligenes*	99.18	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.32	2.81	14	*Pseudomonas kilonensis*, *Pseudomonas jessenii*, *Pseudomonas mohnii*, *Pseudomonas corrugata*, *Pseudomonas vancouverensis*, *Pseudomonas moorei*	100.0	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.886	6.19	45	No hits of at least 97% identity	94.15	*Proteobacteria Gammaproteobacteria WN-HWB-116*
OTU.77	5.4	45	*Pseudoxanthomonas dokdonensis*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.144	4.69	14	No hits of at least 97% identity	95.72	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.499	4.61	28	No hits of at least 97% identity	94.65	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.1178	3.93	28	*Dokdonella sp. KIS28-6*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.3336	3.83	45	No hits of at least 97% identity	92.78	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.230	3.83	45	No hits of at least 97% identity	96.79	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.769	3.82	14	*Arenimonas sp. CH15-1*	97.33	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.109	3.8	28	No hits of at least 97% identity	96.26	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.1574	3.74	45	No hits of at least 97% identity	91.67	*Proteobacteria Gammaproteobacteria Xanthomonadales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.7596	3.63	14	No hits of at least 97% identity	96.42	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.6983	3.61	14	*Dyella koreensis*, *Dyella soli*	97.3	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.51	3.6	28	*Lysobacter gummosus*, *Lysobacter antibioticus*	99.47	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.413	3.5	14	No hits of at least 97% identity	96.52	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.695	3.38	14	*Lysobacter niabensis*	98.4	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.514	3.13	28	No hits of at least 97% identity	96.79	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.8067	2.83	45	No hits of at least 97% identity	88.5	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.79	2.72	45	No hits of at least 97% identity	90.11	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.28	2.61	14	*Lysobacter oryzae*	99.47	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.24	2.15	45	*Arenimonas sp. CH15-1*	98.66	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.1306	5.2	45	No hits of at least 97% identity	85.11	*Verrucomicrobia Candidatus-Methylacidiphilum uncultured-bacterium*
OTU.1632	4.85	45	No hits of at least 97% identity	85.09	*Verrucomicrobia OPB35-soil-group uncultured-bacterium*
OTU.620	4.33	45	No hits of at least 97% identity	83.78	*Verrucomicrobia OPB35-soil-group uncultured-bacterium*
OTU.9695	3.41	45	No hits of at least 97% identity	86.02	*Verrucomicrobia OPB35-soil-group uncultured-bacterium*
OTU.308	3.17	14	No hits of at least 97% identity	84.8	*Verrucomicrobia OPB35-soil-group uncultured-bacterium*
OTU.875	5.71	45	No hits of at least 97% identity	88.06	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.554	5.65	45	No hits of at least 97% identity	85.56	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1237	5.16	45	No hits of at least 97% identity	88.53	*Verrucomicrobia Spartobacteria Chthoniobacterales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.805	5.0	45	No hits of at least 97% identity	89.12	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.752	4.87	45	No hits of at least 97% identity	88.83	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1544	4.8	45	No hits of at least 97% identity	89.66	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.7170	4.48	45	No hits of at least 97% identity	89.52	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.2863	4.38	45	No hits of at least 97% identity	85.16	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.4740	4.37	45	No hits of at least 97% identity	82.38	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1150	4.12	45	No hits of at least 97% identity	85.94	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.473	4.11	45	No hits of at least 97% identity	89.84	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.3106	3.97	28	No hits of at least 97% identity	87.23	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.799	3.9	45	No hits of at least 97% identity	87.57	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.7396	3.54	45	No hits of at least 97% identity	89.34	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.13967	3.4	45	No hits of at least 97% identity	87.36	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.405	3.33	45	No hits of at least 97% identity	87.83	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.327	3.31	45	No hits of at least 97% identity	89.66	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.867	2.54	45	No hits of at least 97% identity	86.1	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.950	7.65	45	*Verrucomicrobiaceae bacterium DC2a-G7*	100.0	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.11380	6.28	45	*Verrucomicrobiaceae bacterium DC2a-G7*	97.28	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.2818	6.25	45	No hits of at least 97% identity	85.56	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.1787	5.59	45	*Luteolibacter pohnpeiensis*, *Luteolibacter sp. CCTCC AB 2010415*	97.59	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.903	5.51	45	No hits of at least 97% identity	94.92	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.5545	5.29	45	*Verrucomicrobium spinosum*	97.84	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*

Table 4.2 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.866	5.13	45	*Verrucomicrobium spinosum*	98.93	*Verrucomicrobia* *Verrucomicrobiae* *Verrucomicrobiales*
OTU.5228	2.65	14	No hits of at least 97% identity	89.73	*Verrucomicrobia* *Verrucomicrobiae* *Verrucomicrobiales*

[a] Maximum observed log_2 of fold change.
[b] Day of maximum fold change.
[c] Against Living Tree Project database.
[d] Annotation from Silva database assigned during OTU binning (see methods).

Table 4.3: ^{13}C-cellulose responders in the repeated root exudate treatment

OTU ID	**Fold change** [a]	**Day** [b]	**Top BLAST hits** [c]	**BLAST %ID** [c]	**Phylum;Class;Order** [d]
OTU.406	2.02	45	No hits of at least 97% identity	85.83	*Acidobacteria DA023 uncultured-bacterium*
OTU.205	1.91	45	No hits of at least 97% identity	91.94	*Acidobacteria Order-Incertae-Sedis Family-Incertae-Sedis*
OTU.7984	4.42	14	No hits of at least 97% identity	89.1	*Actinobacteria Corynebacteriales uncultured*
OTU.8951	5.76	14	No hits of at least 97% identity	93.83	*Actinobacteria Micrococcales Micrococcaceae*
OTU.9722	4.82	14	No hits of at least 97% identity	95.7	*Actinobacteria Streptomycetales Streptomycetaceae*
OTU.1811	4.72	28	No hits of at least 97% identity	92.29	*Armatimonadetes Armatimonadia Armatimonadales*
OTU.998	4.14	28	No hits of at least 97% identity	92.53	*Armatimonadetes Armatimonadia Armatimonadales*
OTU.2526	4.45	28	No hits of at least 97% identity	88.53	*Armatimonadetes Chthonomonadetes Chthonomonadales*
OTU.982	2.53	28	No hits of at least 97% identity	84.8	*Armatimonadetes Chthonomonadetes Chthonomonadales*
OTU.782	5.47	28	No hits of at least 97% identity	94.86	*Bacteroidetes Cytophagia Cytophagales*
OTU.7433	4.79	14	No hits of at least 97% identity	93.52	*Bacteroidetes Cytophagia Cytophagales*
OTU.3926	4.74	28	No hits of at least 97% identity	95.41	*Bacteroidetes Cytophagia Cytophagales*
OTU.774	4.49	45	No hits of at least 97% identity	93.19	*Bacteroidetes Cytophagia Cytophagales*
OTU.911	3.96	14	No hits of at least 97% identity	93.51	*Bacteroidetes Cytophagia Cytophagales*
OTU.645	2.54	28	No hits of at least 97% identity	89.76	*Bacteroidetes Cytophagia Cytophagales*
OTU.13290	5.16	28	*Flavobacterium sp. FCS-5*	98.38	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.750	3.58	28	No hits of at least 97% identity	88.65	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.2137	5.98	28	No hits of at least 97% identity	95.95	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.2215	4.66	28	No hits of at least 97% identity	94.88	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1119	4.56	45	*Flavihumibacter petaseus*	97.84	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1837	4.53	45	No hits of at least 97% identity	93.5	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.838	3.91	45	No hits of at least 97% identity	94.91	*Bacteroidetes Sphingobacteriia Sphingobacteriales*

Table 4.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.632	2.8	45	No hits of at least 97% identity	96.76	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.6354	2.79	45	No hits of at least 97% identity	88.65	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.403	2.6	28	No hits of at least 97% identity	96.76	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1784	3.83	14	No hits of at least 97% identity	83.24	*Candidate-division-BRC1 uncultured-bacterium*
OTU.1783	3.81	14	No hits of at least 97% identity	83.82	*Candidate-division-BRC1 uncultured-bacterium*
OTU.692	3.23	14	No hits of at least 97% identity	85.91	*Chlorobi Chlorobia Chlorobiales*
OTU.597	2.28	28	No hits of at least 97% identity	86.96	*Chlorobi Chlorobia Chlorobiales*
OTU.848	3.83	45	No hits of at least 97% identity	83.65	*Cyanobacteria MLE1-12 uncultured-bacterium*
OTU.1992	7.28	14	No hits of at least 97% identity	93.24	*Cyanobacteria SM1D11 uncultured-bacterium*
OTU.1776	2.87	28	No hits of at least 97% identity	80.76	*Firmicutes Erysipelotrichi Erysipelotrichales*
OTU.2568	4.16	28	No hits of at least 97% identity	86.6	*Gemmatimonadetes Gemmatimonadales Gemmatimonadaceae*
OTU.8775	3.78	28	No hits of at least 97% identity	81.92	*Planctomycetes OM190 uncultured-bacterium*
OTU.1312	3.36	14	No hits of at least 97% identity	81.82	*Planctomycetes OM190 uncultured-bacterium*
OTU.2087	4.51	28	No hits of at least 97% identity	91.15	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1667	4.46	45	No hits of at least 97% identity	88.17	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.12128	4.26	28	No hits of at least 97% identity	93.46	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.6253	4.02	14	No hits of at least 97% identity	85.83	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.5851	3.98	14	No hits of at least 97% identity	88.3	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.2023	3.04	45	No hits of at least 97% identity	90.32	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1310	2.82	28	No hits of at least 97% identity	91.2	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1586	2.67	28	No hits of at least 97% identity	90.62	*Planctomycetes Planctomycetacia Planctomycetales*

Table 4.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.3271	2.66	28	No hits of at least 97% identity	91.14	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.252	2.46	28	No hits of at least 97% identity	90.08	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.209	2.09	28	No hits of at least 97% identity	93.32	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.10671	4.78	28	No hits of at least 97% identity	94.74	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.7576	4.63	14	No hits of at least 97% identity	91.67	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1850	4.59	28	No hits of at least 97% identity	92.29	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.787	3.62	45	*Starkeya koreensis*	98.12	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.4411	3.02	45	No hits of at least 97% identity	92.47	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.223	1.88	45	*Reyranella massiliensis*	97.85	*Proteobacteria Alphaproteobacteria Rhodospirillales*
OTU.6520	5.18	28	No hits of at least 97% identity	84.76	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.1125	3.94	14	No hits of at least 97% identity	84.72	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.1708	5.89	14	No hits of at least 97% identity	94.64	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.6552	5.45	28	*Sphingobium sp. 301*, *Sphingobium amiense*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.9555	4.9	45	*Sphingomonas sp. LNB2*	97.05	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.1196	4.12	45	*Sphingopyxis taejonensis*	100.0	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.4255	5.59	14	No hits of at least 97% identity	92.76	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.8266	3.53	14	No hits of at least 97% identity	93.22	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.2243	2.83	28	*Comamonas koreensis*	98.66	*Proteobacteria Betaproteobacteria Burkholderiales*

Table 4.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.5915	3.19	14	No hits of at least 97% identity	90.32	*Proteobacteria Betaproteobacteria Nitrosomonadales*
OTU.4552	3.24	14	No hits of at least 97% identity	89.67	*Proteobacteria Betaproteobacteria SC-I-84*
OTU.491	3.71	45	No hits of at least 97% identity	89.54	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.1960	3.25	45	No hits of at least 97% identity	90.59	*Proteobacteria Deltaproteobacteria Bdellovibrionales*
OTU.2140	3.97	45	No hits of at least 97% identity	83.29	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.5550	4.05	45	No hits of at least 97% identity	92.76	*Proteobacteria Gammaproteobacteria Alteromonadales*
OTU.1987	5.72	45	No hits of at least 97% identity	92.78	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.2059	4.84	28	No hits of at least 97% identity	94.64	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.3050	4.78	28	No hits of at least 97% identity	88.8	*Proteobacteria Gammaproteobacteria NKB5*
OTU.1212	4.41	14	No hits of at least 97% identity	87.94	*Proteobacteria Gammaproteobacteria NKB5*
OTU.3534	4.2	14	No hits of at least 97% identity	86.74	*Proteobacteria Gammaproteobacteria NKB5*
OTU.610	7.21	45	*Pseudoxanthomonas mexicana*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.1774	5.37	14	No hits of at least 97% identity	94.09	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.6017	3.82	14	No hits of at least 97% identity	93.55	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.6823	3.5	45	*Lysobacter sp. DCY21T*	97.83	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.11115	2.88	14	No hits of at least 97% identity	94.92	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.193	2.81	28	*Lysobacter ginsengisoli*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.4934	2.45	14	*Xanthomonas sp. T7-07*	99.47	*Proteobacteria Gammaproteobacteria Xanthomonadales*

Table 4.3 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.414	2.18	45	*Stenotrophomonas pavanii*, *Stenotrophomonas maltophilia*	100.0	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.303	2.15	14	*Arenimonas sp. CH15-1*	98.13	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.5763	2.09	45	*Thermomonas dokdonensis*	98.92	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.5454	2.09	14	*Arenimonas sp. CH15-1*	97.59	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.8860	3.96	28	No hits of at least 97% identity	88.15	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1553	5.34	45	No hits of at least 97% identity	96.79	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*

[a] Maximum observed log_2 of fold change.
[b] Day of maximum fold change.
[c] Against Living Tree Project database.
[d] Annotation from Silva database assigned during OTU binning (see methods).

Table 4.4: ^{13}C-cellulose responders in the pulse root exudate treatment

OTU ID	**Fold change** [a]	**Day** [b]	**Top BLAST hits** [c]	**BLAST %ID** [c]	**Phylum;Class;Order** [d]
OTU.1341	4.77	14	No hits of at least 97% identity	86.02	*Acidobacteria 11-24 uncultured-bacterium*
OTU.2288	3.64	45	No hits of at least 97% identity	82.7	*Acidobacteria 11-24 uncultured-bacterium*
OTU.1197	2.41	45	No hits of at least 97% identity	83.02	*Acidobacteria 11-24 uncultured-bacterium*
OTU.1507	3.24	45	No hits of at least 97% identity	82.24	*Acidobacteria Candidatus-Chloracidobacterium uncultured-Acidobacteria-bacterium*
OTU.3817	2.44	28	No hits of at least 97% identity	86.36	*Acidobacteria DA023 uncultured-bacterium*
OTU.12188	6.09	45	No hits of at least 97% identity	86.44	*Acidobacteria Holophagae CA002*
OTU.1016	4.18	14	No hits of at least 97% identity	85.18	*Acidobacteria RB25 uncultured-bacterium*
OTU.370	4.37	28	*Gordonia sputi*	100.0	*Actinobacteria Corynebacteriales Nocardiaceae*
OTU.1181	4.79	14	No hits of at least 97% identity	92.27	*Armatimonadetes Armatimonadia Armatimonadales*
OTU.1303	4.57	45	No hits of at least 97% identity	82.34	*Armatimonadetes uncultured-bacterium*
OTU.1664	7.31	14	*Sporocytophaga myxococcoides*	99.19	*Bacteroidetes Cytophagia Cytophagales*
OTU.1026	4.75	14	No hits of at least 97% identity	94.32	*Bacteroidetes Cytophagia Cytophagales*
OTU.2905	4.2	45	No hits of at least 97% identity	91.85	*Bacteroidetes Cytophagia Cytophagales*
OTU.7868	3.58	14	No hits of at least 97% identity	96.94	*Bacteroidetes Cytophagia Cytophagales*
OTU.331	3.32	14	*Ohtaekwangia kribbensis*	97.04	*Bacteroidetes Cytophagia Cytophagales*
OTU.10192	3.07	14	*Adhaeribacter terreus*	97.57	*Bacteroidetes Cytophagia Cytophagales*
OTU.6036	3.02	14	*Ohtaekwangia kribbensis*	100.0	*Bacteroidetes Cytophagia Cytophagales*
OTU.598	2.94	14	*Sphingobacteria bacterium RYG*	97.31	*Bacteroidetes Cytophagia Cytophagales*
OTU.152	2.53	14	*Adhaeribacter terreus*	98.11	*Bacteroidetes Cytophagia Cytophagales*
OTU.155	2.09	14	No hits of at least 97% identity	92.7	*Bacteroidetes Cytophagia Cytophagales*
OTU.44	2.04	14	No hits of at least 97% identity	95.14	*Bacteroidetes Cytophagia Cytophagales*
OTU.2099	4.99	45	*Chryseobacterium sp. THG 15*, *Chryseobacterium formosense*	100.0	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.1330	4.7	14	*Flavobacterium fluvii*	99.73	*Bacteroidetes Flavobacteria Flavobacteriales*

Table 4.4 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.7315	2.72	14	*Flavobacterium columnare*	98.84	*Bacteroidetes Flavobacteria Flavobacteriales*
OTU.2082	6.2	28	No hits of at least 97% identity	86.7	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1414	4.85	14	No hits of at least 97% identity	89.49	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1929	4.8	14	No hits of at least 97% identity	96.49	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.4996	4.2	14	No hits of at least 97% identity	96.22	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.283	4.11	14	No hits of at least 97% identity	94.32	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.873	3.95	14	*Segetibacter aerophilus*	97.03	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.140	3.89	14	No hits of at least 97% identity	95.14	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.1969	3.67	14	No hits of at least 97% identity	96.76	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.325	3.59	14	*Flavisolibacter ginsengisoli*	98.38	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.490	3.5	14	No hits of at least 97% identity	95.41	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.425	3.38	14	No hits of at least 97% identity	88.44	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.3332	3.38	14	No hits of at least 97% identity	96.76	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.343	3.33	14	No hits of at least 97% identity	92.45	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.3380	3.28	14	No hits of at least 97% identity	96.22	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.717	3.24	14	No hits of at least 97% identity	89.62	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7661	3.17	14	No hits of at least 97% identity	95.69	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7953	3.07	14	No hits of at least 97% identity	94.59	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.178	3.07	14	No hits of at least 97% identity	94.86	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.145	3.02	14	No hits of at least 97% identity	90.84	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.718	3.02	14	No hits of at least 97% identity	88.56	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7103	3.0	14	No hits of at least 97% identity	87.87	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.215	2.93	14	No hits of at least 97% identity	96.49	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.7560	2.91	14	*Pedobacter africanus*, *Pedobacter steynii*, *Pedobacter caeni*	98.65	*Bacteroidetes Sphingobacteriia Sphingobacteriales*

Table 4.4 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.12538	2.83	14	No hits of at least 97% identity	93.5	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.523	2.83	14	No hits of at least 97% identity	88.65	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.103	2.83	14	No hits of at least 97% identity	95.95	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.9220	2.8	14	No hits of at least 97% identity	94.86	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.3730	2.74	14	No hits of at least 97% identity	95.41	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.498	2.72	14	No hits of at least 97% identity	88.38	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.570	2.66	14	No hits of at least 97% identity	92.18	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.5218	2.6	14	No hits of at least 97% identity	95.14	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.431	2.6	14	No hits of at least 97% identity	93.55	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.916	2.6	28	*Chitinophaga niabensis*	97.57	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.75	2.54	14	*Flavisolibacter ginsengisoli*	97.84	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.45	2.51	14	*Flavitalea populi*	98.92	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.228	2.5	14	No hits of at least 97% identity	94.59	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.3841	2.43	14	No hits of at least 97% identity	93.24	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.133	2.36	14	No hits of at least 97% identity	95.41	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.725	2.34	14	No hits of at least 97% identity	95.95	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.225	2.33	14	No hits of at least 97% identity	96.49	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.8100	2.13	14	No hits of at least 97% identity	95.52	*Bacteroidetes Sphingobacteriia Sphingobacteriales*
OTU.2351	5.22	14	No hits of at least 97% identity	88.59	*Bacteroidetes VC2.1-Bac22 uncultured-bacterium*
OTU.3465	4.28	45	No hits of at least 97% identity	75.53	*Candidate-division-OP11 uncultured-bacterium*
OTU.1980	3.97	14	No hits of at least 97% identity	85.91	*Candidate-division-WS3 uncultured-bacterium*
OTU.3682	2.63	14	No hits of at least 97% identity	83.96	*Candidate-division-WS3 uncultured-bacterium*
OTU.509	2.24	14	No hits of at least 97% identity	85.98	*Candidate-division-WS3 uncultured-bacterium*
OTU.958	4.72	14	No hits of at least 97% identity	82.61	*Chlorobi Chlorobia Chlorobiales*

Table 4.4 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.1217	3.2	45	No hits of at least 97% identity	80.48	*Chloroflexi Anaerolineae Anaerolineales*
OTU.907	2.83	14	No hits of at least 97% identity	83.68	*Chloroflexi Anaerolineae Anaerolineales*
OTU.3983	6.24	45	No hits of at least 97% identity	80.31	*Chloroflexi Chloroflexales Chloroflexaceae*
OTU.3585	5.14	45	No hits of at least 97% identity	96.22	*Cyanobacteria SM1D11*
OTU.640	3.86	28	*Streptococcus tigurinus*, *Streptococcus mitis*, *Streptococcus infantis*, *Streptococcus pseudopneumoniae*, *Streptococcus oralis*	99.73	*Firmicutes Bacilli Lactobacillales*
OTU.1115	3.0	28	No hits of at least 97% identity	80.59	*Planctomycetes Phycisphaerae WD2101-soil-group*
OTU.5726	5.13	14	No hits of at least 97% identity	90.4	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.6106	4.63	45	No hits of at least 97% identity	92.27	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.3395	3.53	45	No hits of at least 97% identity	85.25	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.7980	3.39	45	No hits of at least 97% identity	94.13	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.1073	2.93	45	No hits of at least 97% identity	89.63	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.6068	2.83	14	No hits of at least 97% identity	85.79	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.345	2.04	14	No hits of at least 97% identity	89.52	*Planctomycetes Planctomycetacia Planctomycetales*
OTU.3431	3.78	28	No hits of at least 97% identity	87.17	*Proteobacteria Alphaproteobacteria DB1-14*
OTU.7792	5.13	14	No hits of at least 97% identity	90.35	*Proteobacteria Alphaproteobacteria OCS116-clade*
OTU.6486	3.97	45	*Kaistia sp. B1-1*	98.92	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.991	3.21	14	*Kaistia sp. 5YN7-3*, *Kaistia sp. B6-12*	100.0	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.517	2.66	14	No hits of at least 97% identity	94.1	*Proteobacteria Alphaproteobacteria Rhizobiales*
OTU.1627	5.95	45	No hits of at least 97% identity	91.76	*Proteobacteria Alphaproteobacteria Rhodospirillales*

Table 4.4 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.187	4.08	28	*Roseomonas gilardii subsp. gilardii*, *Roseomonas mucosa*, *Roseomonas gilardii subsp. rosea*	100.0	*Proteobacteria Alphaproteobacteria Rhodospirillales*
OTU.482	2.22	14	No hits of at least 97% identity	90.45	*Proteobacteria Alphaproteobacteria Rhodospirillales*
OTU.7443	2.07	28	No hits of at least 97% identity	95.38	*Proteobacteria Alphaproteobacteria Rhodospirillales*
OTU.2141	5.38	14	No hits of at least 97% identity	84.49	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.486	5.31	14	No hits of at least 97% identity	90.03	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.1930	4.67	14	No hits of at least 97% identity	87.77	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.1165	3.99	14	No hits of at least 97% identity	88.47	*Proteobacteria Alphaproteobacteria Rickettsiales*
OTU.2814	5.28	14	No hits of at least 97% identity	96.26	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.289	2.61	14	No hits of at least 97% identity	96.78	*Proteobacteria Alphaproteobacteria Sphingomonadales*
OTU.141	4.69	14	No hits of at least 97% identity	94.65	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.5640	3.4	45	*Paucimonas lemoignei*	98.12	*Proteobacteria Betaproteobacteria Burkholderiales*
OTU.2239	4.22	45	No hits of at least 97% identity	94.12	*Proteobacteria Betaproteobacteria Hydrogenophilales*
OTU.3811	4.57	14	No hits of at least 97% identity	89.49	*Proteobacteria Deltaproteobacteria Desulfobacterales*
OTU.2036	4.69	45	No hits of at least 97% identity	87.09	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.696	2.34	45	No hits of at least 97% identity	93.85	*Proteobacteria Deltaproteobacteria Myxococcales*
OTU.6577	5.83	45	No hits of at least 97% identity	95.72	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.1190	4.28	45	No hits of at least 97% identity	96.27	*Proteobacteria Gammaproteobacteria Legionellales*

Table 4.4 – continued from previous page

OTU ID	Fold change	Day	Top BLAST hits	BLAST %ID	Phylum;Class;Order
OTU.475	2.28	45	*Aquicella siphonis*	97.31	*Proteobacteria Gammaproteobacteria Legionellales*
OTU.433	5.63	45	No hits of at least 97% identity	87.47	*Proteobacteria Gammaproteobacteria NKB5*
OTU.2651	4.31	45	No hits of at least 97% identity	87.4	*Proteobacteria Gammaproteobacteria NKB5*
OTU.10253	4.48	14	No hits of at least 97% identity	93.26	*Proteobacteria Gammaproteobacteria Pseudomonadales*
OTU.1332	4.12	14	*Panacagrimonas perspica*	97.86	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.273	2.16	14	No hits of at least 97% identity	94.88	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.465	2.15	14	No hits of at least 97% identity	94.34	*Proteobacteria Gammaproteobacteria Xanthomonadales*
OTU.5114	6.24	14	No hits of at least 97% identity	92.78	*Verrucomicrobia Opitutae Opitutales*
OTU.4224	5.35	45	No hits of at least 97% identity	88.1	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.1993	3.77	45	No hits of at least 97% identity	88.1	*Verrucomicrobia Spartobacteria Chthoniobacterales*
OTU.10213	4.65	28	*Prosthecobacter fusiformis*	98.4	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.3003	4.31	45	*Prosthecobacter fluviatilis*	97.33	*Verrucomicrobia Verrucomicrobiae Verrucomicrobiales*
OTU.1705	3.06	45	No hits of at least 97% identity	84.76	

[a] Maximum observed log_2 of fold change.
[b] Day of maximum fold change.
[c] Against Living Tree Project database.
[d] Annotation from Silva database assigned during OTU binning (see methods).

Table 4.5 Cellulose responders identified exclusively in only one treatment

OTU	Treatment	Phylum	Class	Order
OTU.1707	Cellulose Only	Acidobacteria	Holophagae	CA002
OTU.7337	Cellulose Only	Acidobacteria	Holophagae	iii1-8
OTU.1576	Cellulose Only	Acidobacteria	Order_Incertae_Sedis	Family_Incertae_Sedis
OTU.1	Cellulose Only	Actinobacteria	Micrococcales	Micrococcaceae
OTU.347	Cellulose Only	Actinobacteria	Micromonosporales	Micromonosporaceae
OTU.2360	Cellulose Only	Actinobacteria	Micromonosporales	Micromonosporaceae
OTU.1598	Cellulose Only	Actinobacteria	Propionibacteriales	Nocardioidaceae
OTU.567	Cellulose Only	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae
OTU.11083	Cellulose Only	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae
OTU.101	Cellulose Only	Actinobacteria	Streptomycetales	Streptomycetaceae
OTU.1219	Cellulose Only	Armatimonadetes	uncultured_bacterium	NA
OTU.10789	Cellulose Only	Bacteroidetes	Cytophagia	Cytophagales
OTU.1647	Cellulose Only	Bacteroidetes	Cytophagia	Cytophagales
OTU.2730	Cellulose Only	Bacteroidetes	Cytophagia	Cytophagales
OTU.1847	Cellulose Only	Bacteroidetes	Cytophagia	Cytophagales
OTU.1171	Cellulose Only	Bacteroidetes	Flavobacteria	Flavobacteriales
OTU.12560	Cellulose Only	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.1862	Cellulose Only	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.877	Cellulose Only	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.2157	Cellulose Only	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.5258	Cellulose Only	BD1-5	uncultured_bacterium	NA
OTU.694	Cellulose Only	Candidate_division_BRC1	uncultured_bacterium	NA
OTU.2028	Cellulose Only	Candidate_division_OD1	uncultured_bacterium	NA
OTU.941	Cellulose Only	Chlorobi	Chlorobia	Chlorobiales
OTU.1551	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.1703	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.2050	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.2440	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.3038	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.8564	Cellulose Only	Chloroflexi	Anaerolineae	Anaerolineales
OTU.1629	Cellulose Only	Cyanobacteria	MLE1-12	uncultured_bacterium
OTU.5011	Cellulose Only	Cyanobacteria	SM1D11	uncultured_bacterium
OTU.3573	Cellulose Only	Elusimicrobia	Lineage_IV	uncultured_bacterium
OTU.6775	Cellulose Only	Elusimicrobia	MVP-88	NA
OTU.1102	Cellulose Only	Gemmatimonadetes	AT425-EubC11_terrestrial_group	uncultured_bacterium
OTU.763	Cellulose Only	Nitrospirae	Nitrospira	Nitrospirales
OTU.494	Cellulose Only	Planctomycetes	OM190	NA
OTU.1029	Cellulose Only	Planctomycetes	OM190	uncultured_bacterium

OTU.549	Cellulose Only	Planctomycetes	OM190	uncultured_bacterium
OTU.1077	Cellulose Only	Planctomycetes	Phycisphaerae	Phycisphaerales
OTU.3073	Cellulose Only	Planctomycetes	Phycisphaerae	Phycisphaerales
OTU.1324	Cellulose Only	Planctomycetes	Phycisphaerae	WD2101_soil_group
OTU.3000	Cellulose Only	Planctomycetes	Phycisphaerae	WD2101_soil_group
OTU.307	Cellulose Only	Planctomycetes	Phycisphaerae	WD2101_soil_group
OTU.1174	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1239	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1338	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1702	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2102	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.213	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2151	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2441	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2880	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2901	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.3091	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.3667	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.368	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.3835	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.445	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.4923	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.559	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.685	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.825	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.8906	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.9627	Cellulose Only	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1329	Cellulose Only	Planctomycetes	vadinHA49	uncultured_bacterium
OTU.12989	Cellulose Only	Proteobacteria	Alphaproteobacteria	Caulobacterales
OTU.3030	Cellulose Only	Proteobacteria	Alphaproteobacteria	Caulobacterales
OTU.566	Cellulose Only	Proteobacteria	Alphaproteobacteria	Caulobacterales
OTU.5106	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.6217	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.6819	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.512	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.264	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.107	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.11661	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.766	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.2418	Cellulose Only	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.13	Cellulose Only	Proteobacteria	Alphaproteobacteria	Sphingomonadales

OTU.3156	Cellulose Only	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.5320	Cellulose Only	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.6783	Cellulose Only	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.9664	Cellulose Only	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.5914	Cellulose Only	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.4112	Cellulose Only	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.441	Cellulose Only	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.5544	Cellulose Only	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.815	Cellulose Only	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.2161	Cellulose Only	Proteobacteria	Betaproteobacteria	Hydrogenophilales
OTU.1967	Cellulose Only	Proteobacteria	Betaproteobacteria	Rhodocyclales
OTU.2276	Cellulose Only	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
OTU.2525	Cellulose Only	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
OTU.4322	Cellulose Only	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
OTU.4156	Cellulose Only	Proteobacteria	Deltaproteobacteria	GR-WP33-30
OTU.1097	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.1736	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.2610	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.3805	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.1900	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.927	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.1573	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.2624	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.2628	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.3831	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.3842	Cellulose Only	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.1398	Cellulose Only	Proteobacteria	Deltaproteobacteria	Sh765B-TzT-29
OTU.1318	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.2212	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.2329	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.2386	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.3267	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.811	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.2213	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.3661	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.4746	Cellulose Only	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.3310	Cellulose Only	Proteobacteria	Gammaproteobacteria	Pseudomonadales
OTU.32	Cellulose Only	Proteobacteria	Gammaproteobacteria	Pseudomonadales
OTU.3336	Cellulose Only	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.514	Cellulose Only	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.1632	Cellulose Only	Verrucomicrobia	OPB35_soil_group	uncultured_bacterium

OTU.308	Cellulose Only	Verrucomicrobia	OPB35_soil_group	uncultured_bacterium
OTU.620	Cellulose Only	Verrucomicrobia	OPB35_soil_group	uncultured_bacterium
OTU.9695	Cellulose Only	Verrucomicrobia	OPB35_soil_group	uncultured_bacterium
OTU.2863	Cellulose Only	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.13967	Cellulose Only	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.799	Cellulose Only	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.867	Cellulose Only	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.3106	Cellulose Only	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.5228	Cellulose Only	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
OTU.406	RE - Repeat	Acidobacteria	DA023	uncultured_bacterium
OTU.205	RE - Repeat	Acidobacteria	Order_Incertae_Sedis	Family_Incertae_Sedis
OTU.7984	RE - Repeat	Actinobacteria	Corynebacteriales	uncultured
OTU.9722	RE - Repeat	Actinobacteria	Streptomycetales	Streptomycetaceae
OTU.1811	RE - Repeat	Armatimonadetes	Armatimonadia	Armatimonadales
OTU.2526	RE - Repeat	Armatimonadetes	Chthonomonadetes	Chthonomonadales
OTU.982	RE - Repeat	Armatimonadetes	Chthonomonadetes	Chthonomonadales
OTU.3926	RE - Repeat	Bacteroidetes	Cytophagia	Cytophagales
OTU.13290	RE - Repeat	Bacteroidetes	Flavobacteria	Flavobacteriales
OTU.1837	RE - Repeat	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.2137	RE - Repeat	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.2215	RE - Repeat	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.403	RE - Repeat	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.1783	RE - Repeat	Candidate_division_BRC1	uncultured_bacterium	NA
OTU.597	RE - Repeat	Chlorobi	Chlorobia	Chlorobiales
OTU.692	RE - Repeat	Chlorobi	Chlorobia	Chlorobiales
OTU.848	RE - Repeat	Cyanobacteria	MLE1-12	uncultured_bacterium
OTU.1992	RE - Repeat	Cyanobacteria	SM1D11	uncultured_bacterium
OTU.2568	RE - Repeat	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
OTU.1312	RE - Repeat	Planctomycetes	OM190	uncultured_bacterium
OTU.12128	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1310	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.1586	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.2023	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.5851	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.6253	RE - Repeat	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.7576	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.1850	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.10671	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.787	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.223	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rhodospirillales
OTU.6520	RE - Repeat	Proteobacteria	Alphaproteobacteria	Rickettsiales

OTU.1708	RE - Repeat	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.8266	RE - Repeat	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.4255	RE - Repeat	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.5915	RE - Repeat	Proteobacteria	Betaproteobacteria	Nitrosomonadales
OTU.4552	RE - Repeat	Proteobacteria	Betaproteobacteria	SC-I-84
OTU.1960	RE - Repeat	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
OTU.2140	RE - Repeat	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.5550	RE - Repeat	Proteobacteria	Gammaproteobacteria	Alteromonadales
OTU.2059	RE - Repeat	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.1212	RE - Repeat	Proteobacteria	Gammaproteobacteria	NKB5
OTU.3050	RE - Repeat	Proteobacteria	Gammaproteobacteria	NKB5
OTU.3534	RE - Repeat	Proteobacteria	Gammaproteobacteria	NKB5
OTU.11115	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.303	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.414	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.5454	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.5763	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.6017	RE - Repeat	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.8860	RE - Repeat	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.1197	RE - Pulse	Acidobacteria	40870	uncultured_bacterium
OTU.1341	RE - Pulse	Acidobacteria	40870	uncultured_bacterium
OTU.2288	RE - Pulse	Acidobacteria	40870	uncultured_bacterium
OTU.1507	RE - Pulse	Acidobacteria	Candidatus_Chloracidobacterium	uncultured_Acidobacteria_bacterium
OTU.3817	RE - Pulse	Acidobacteria	DA023	uncultured_bacterium
OTU.12188	RE - Pulse	Acidobacteria	Holophagae	CA002
OTU.1016	RE - Pulse	Acidobacteria	RB25	uncultured_bacterium
OTU.370	RE - Pulse	Actinobacteria	Corynebacteriales	Nocardiaceae
OTU.1181	RE - Pulse	Armatimonadetes	Armatimonadia	Armatimonadales
OTU.1303	RE - Pulse	Armatimonadetes	uncultured_bacterium	NA
OTU.10192	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.1026	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.152	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.155	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.1664	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.2905	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.331	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.44	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.598	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.6036	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.7868	RE - Pulse	Bacteroidetes	Cytophagia	Cytophagales
OTU.1330	RE - Pulse	Bacteroidetes	Flavobacteria	Flavobacteriales

OTU.2099	RE - Pulse	Bacteroidetes	Flavobacteria	Flavobacteriales
OTU.7315	RE - Pulse	Bacteroidetes	Flavobacteria	Flavobacteriales
OTU.145	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.717	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.103	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.12538	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.133	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.140	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.178	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.1929	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.1969	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.215	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.225	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.228	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.283	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.325	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.3332	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.3380	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.343	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.3730	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.3841	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.431	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.45	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.490	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.4996	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.5218	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.570	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.725	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.75	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.7661	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.7953	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.8100	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.873	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.916	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.9220	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.2082	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.1414	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.718	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.425	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.498	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.523	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales

OTU.7103	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.7560	RE - Pulse	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
OTU.2351	RE - Pulse	Bacteroidetes	VC2.1_Bac22	uncultured_bacterium
OTU.3465	RE - Pulse	Candidate_division_OP11	uncultured_bacterium	NA
OTU.1980	RE - Pulse	Candidate_division_WS3	uncultured_bacterium	NA
OTU.3682	RE - Pulse	Candidate_division_WS3	uncultured_bacterium	NA
OTU.509	RE - Pulse	Candidate_division_WS3	uncultured_bacterium	NA
OTU.958	RE - Pulse	Chlorobi	Chlorobia	Chlorobiales
OTU.1217	RE - Pulse	Chloroflexi	Anaerolineae	Anaerolineales
OTU.907	RE - Pulse	Chloroflexi	Anaerolineae	Anaerolineales
OTU.3983	RE - Pulse	Chloroflexi	Chloroflexales	Chloroflexaceae
OTU.3585	RE - Pulse	Cyanobacteria	SM1D11	NA
OTU.640	RE - Pulse	Firmicutes	Bacilli	Lactobacillales
OTU.1705	RE - Pulse	NA	NA	NA
OTU.1115	RE - Pulse	Planctomycetes	Phycisphaerae	WD2101_soil_group
OTU.1073	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.3395	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.345	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.5726	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.6068	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.6106	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.7980	RE - Pulse	Planctomycetes	Planctomycetacia	Planctomycetales
OTU.3431	RE - Pulse	Proteobacteria	Alphaproteobacteria	DB1-14
OTU.7792	RE - Pulse	Proteobacteria	Alphaproteobacteria	OCS116_clade
OTU.6486	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.991	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.517	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhizobiales
OTU.187	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhodospirillales
OTU.482	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhodospirillales
OTU.1627	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhodospirillales
OTU.7443	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rhodospirillales
OTU.1165	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.1930	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.486	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.2141	RE - Pulse	Proteobacteria	Alphaproteobacteria	Rickettsiales
OTU.289	RE - Pulse	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.2814	RE - Pulse	Proteobacteria	Alphaproteobacteria	Sphingomonadales
OTU.141	RE - Pulse	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.5640	RE - Pulse	Proteobacteria	Betaproteobacteria	Burkholderiales
OTU.2239	RE - Pulse	Proteobacteria	Betaproteobacteria	Hydrogenophilales

OTU.3811	RE - Pulse	Proteobacteria	Deltaproteobacteria	Desulfobacterales
OTU.2036	RE - Pulse	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.696	RE - Pulse	Proteobacteria	Deltaproteobacteria	Myxococcales
OTU.475	RE - Pulse	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.1190	RE - Pulse	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.6577	RE - Pulse	Proteobacteria	Gammaproteobacteria	Legionellales
OTU.2651	RE - Pulse	Proteobacteria	Gammaproteobacteria	NKB5
OTU.433	RE - Pulse	Proteobacteria	Gammaproteobacteria	NKB5
OTU.10253	RE - Pulse	Proteobacteria	Gammaproteobacteria	Pseudomonadales
OTU.1332	RE - Pulse	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.273	RE - Pulse	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.465	RE - Pulse	Proteobacteria	Gammaproteobacteria	Xanthomonadales
OTU.5114	RE - Pulse	Verrucomicrobia	Opitutae	Opitutales
OTU.1993	RE - Pulse	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.4224	RE - Pulse	Verrucomicrobia	Spartobacteria	Chthoniobacterales
OTU.10213	RE - Pulse	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
OTU.3003	RE - Pulse	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales

Conclusion

5.1 Summation

This dissertation examined organic matter decomposition dynamics in soil using a novel approach, high resolution stable isotope probing (HR-SIP). We found the dynamics of carbon (C) use varies with substrate complexity (Chapter 2). We conclude that organic matter decomposition occurs in a succession of phases: substrates of low complexity are decomposed in the first phase and during the second phase, substrates of higher complexity are decomposed. The successive phases of decomposition are accompanied by a succession of different responders. Xylose (soluble, monomer subunit) was decomposed within a week of being added to soils and xylose responders were dynamic exhibiting a succession from *Firmicutes*, to *Bacteroidetes*, to *Actinobacteria*. Cellulose (insoluble, polymer) in contrast, was decomposed more slowly with most responders detected after two weeks and exhibiting a less dynamic response pattern over time; similar responders were detected at 2 and 4 weeks. Cellulose responders were also taxonomically distinct from xylose responders, and they had greater changes in center of mass in density gradients, lower *rrn* copy number, and were less abundant in the community than xylose responders. These findings indicate that the different xylose and cellulose responders have different ecological strategies which govern their responses to the input of new carbon to soil.

Niche partitioning preserves the function of cellulose decomposition in soil (Chapter 3). We found different cellulose responders when soils were amended with

cellulose in the absence or presence of additional nutrients. Despite differences in cellulose responders resulting from amendment composition, there was no difference in the amount of cellulose decomposition. This suggests that the function of cellulose decomposition is preserved under varying environmental conditions by niche partitioning of cellulose responders.

Root exudate additions to soil did not prime cellulose decomposition (Chapter 4). Additionally, cellulose decomposition dynamics occurred in three successive phases regardless of amendment composition, timing of addition or concentration. The first phase had the highest rate of cellulose mineralization (days 8-19) and was characterized by increasing rate of cellulose use, the second phase (days 20-33) was a decline of cellulose mineralization, and the third phase (days 34-47) was characterized by a plateau in the rate of cellulose decomposition, signifying a steady state in the rate of cellulose decomposition. The successive phases of cellulose decomposition are the same despite differences in phylogenetic affiliation of cellulose responders between the treatments and sampling times. Therefore, cellulose decomposition proceeds in a predictable successive process. However, preservation of the cellulose decomposition function in treatments depends on maintaining a diversity of cellulose responders that are active at different environmental conditions.

5.2 The value of high resolution stable isotope probing

Using HR-SIP, we revealed dynamic patterns of xylose and cellulose use by a multitude of microbial community members. The increased resolution HR-SIP is multifaceted and each facet is discussed in greater detail in the following

paragraphs. This approach includes (1) addition of C substrates added in a complex mixture at low (biologically relevant) concentrations, (2) partitioning of the density gradient into multiple, small fractions for a fine scale resolution of density shifts resulting from ^{13}C assimilation, (3) next generation sequencing of individual fractions for greater taxonomic depth, and (4) sampling C-assimilation over a time series in which assimilation of ^{13}C by individual taxa can be observed. These were mentioned as ways to better assess microbial interactions using SIP (Abraham 2014).

SIP experiments have traditionally used high concentrations of substrate addition to ensure detection of the isotope. These additions are not biologically accurate calling into question the environmental relevance of the results. High concentrations of a substrate result in osmotic stress causing induction of the cell's stress response or even lysis (Kempf, Bremer 1998). In addition, C in soil exists as a complex mixture of substrates, not a single C substrate. Therefore, activity of microbes resulting from the addition of C as a single substrate may not represent the activity microbes in the wild. For these reasons, complex mixtures of C substrates were designed for the studies in this dissertation and added at low C concentrations in an attempt to better mimic biologically relevant conditions.

Most SIP studies fractionate their nucleic acid density gradients into 'light' and 'heavy' fractions and compare the abundance of microbes in each of these fractions relative to a control (unlabeled isotope) gradient. This approach is acceptable to identify microorganisms that utilize specific substrates. However, as a means to understanding ecosystem function and soil C-cycling, this approach is

unacceptable. Fractionating our density gradients into multiple, small fractions enables the detection of the population of specific taxa throughout the density gradient in tiny increments. Significant shifts along the density gradient are indicative of ^{13}C-assimilation and allow for hypothesis generation about their biology and role in the community. Smaller shifts (small amount of ^{13}C assimilation) could mean that a microorganism uses multiple substrates (ie. generalist), is a predator, uses secondary metabolites, or the substrate was bioavailable to only part of the community. Larger shifts (large amount of ^{13}C assimilation) could mean that a microorganism preferentially utilizes that substrate or the substrate may be the only substrate bioavailable to microbes in their spatial niche. Information gained from this can aid in targeted approaches for studying specific taxa or functional groups in microbial communities.

In order to link microbial function to identity using SIP, it must be coupled with a downstream molecular technique that enables taxonomic identification. Traditionally SIP studies have used fingerprinting techniques (ie. DGGE, ARISA, or TRFLP) or cloning. These methods are limited by depth and have low taxonomic resolution, meaning that much of the microbial community is not observed. In HR-SIP, each of the multiple, small fractions from each density gradient is tagged and then sequenced using next generation technology. This enables a greater taxonomic resolution throughout the density gradient.

SIP studies traditionally use one time point to measure substrate utilization and very few have followed the change in the microbial community through time (Brant et al., 2006). In order to accurately understand C dynamics, we should be

looking at C-substrate assimilation over time. The reason for this is because multiple microorganisms utilize a substrate, however, they may do so over varying time scales. For example, some microorganisms are able to respond quickly to the addition of nutrients, whereas, other microorganisms may respond slowly. Slow responding microorganisms may be important players in the cycling of the specific substrate being measured, but may not be captured if incubations are terminated too early. For this reason, substrate utilization by a microbial community should be measured over time to fully understand C-cycling dynamics.

In conclusion, the experimental design integrated into HR-SIP creates a means to examine C processes with higher resolution than previously employed in SIP studies.

5.3 Final Remarks

The debate of whether microbial community composition is important to ecosystem processes or not depends on substrate complexity, temporal measurements, and aggregate vs. individual processes. I would hypothesize that microbial community composition matters for some processes and for some it does not – so long as microbial biodiversity is maintained. In order for us to understand which microbial members matter to ecosystem scale functions, we have to identify them by catching them in the act of mineralization for specific substrates. The information presented in this dissertation highlights a need to examine substrate utilization by discrete microbial taxa within a whole community context to better understand how specific community members function within the whole. HR-SIP

provides a means to elucidate substrate utilization by discrete microbial taxa with the hope that we can begin to construct a belowground C food web.

In the future, important considerations would not only be to understand the importance of microbial structure to the rate of decomposition but also the fate of C substrates. Microorganisms can have different C use efficiencies, may transform C-substrates into different metabolites, or exhibit variations in how they allocate C in its biomass, ultimately altering the fate of C. Intrinsic soil properties and the form of C after mineralization determine C residence time in soil.

5.4 References

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